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TITLE: Levels of the Novel Glycoprotein Lacritin in Human Tears After Laser Refractive Surgery

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14. ABSTRACT
Lacritin is a naturally occurring tear protein with antimicrobial activity that is capable of stimulating mitogenesis in human corneal epithelial cells and promoting production of tears in lacrimal gland acinar cells. Heparanase (HPSE) acts as a regulator for lacritin by cleaving heparan sulfate chains and allowi lacritin to bind. We aim to measure both tear lacritin and HPSE pre- and post-operatively to elucidate lacritin and HPSE's response in patients undergoing PR (photorefractive keratectomy) and LASIK (Laser-assisted in situ keratomileusis) with the possibility of the development of recombinant lacritin as a novel therapeutic agent for wound healing. Up to 196 patients eligible to undergo PRK or LASIK at the Warfighter Refractive Surgery and Research Center at Fort Belvoir will be consecutively recruited: 98 PRK (49male;49female) and 98 LASIK (49male;49 female). Tears will be collected using a safe and established method at the pre-operative visit and at 1 day, 1 week, 1, 3 and 6 months post-operatively to quantify tear lacritin and HPSE. Study design will allow for with subject comparison of lacritin and HPSE before and after surgery as well as comparison of responses between procedures (PRK vs. LASIK). The primary outcome measure is tear lacritin levels pre- and post-surgery. The secondary outcome is tear HPSE levels pre-and post-surgery. Preliminary data in PRK participants shows there is a significant difference in lacritin levels within in the early postoperative period and between surgical procedures. It is unknown whether such differences would have a meaningful impact on visual outcomes or optical quality. Based on the PRK study results to date, there is no correlati Ongoing testing in this study will help determine if there is a difference in lacritin levels in participants undergoing LASIK as well as if lacritin concentration affect early and long-term optical quality and visual outcomes in PRK and LASIK participants.

15. SUBJECT TERMS
Refractive Surgery, PRK, LASIK, lacritin, heparanase, dry eye, optical quality

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INTRODUCTION

Laser refractive surgery is one the most commonly performed elective surgery worldwide. Two well-known procedures are laser in situ keratomileusis (LASIK) and photorefractive keratectomy (PRK). Both procedures are proven safe and effective in correcting nearsightedness, farsightedness and astigmatism.

Like in any other surgical procedure, complications may arise from LASIK and PRK. Dry eye is a well-recognized complication of laser refractive surgery. Post-refractive surgery dry eye may range from mild and transient to severe and persistent condition [Nettune et al. 2010]. Previous studies suggest LASIK produce a greater damage to the corneal nerves that drive tear production and secretion [Ang et al. 2001], thus dry eye may be more prevalent in LASIK than in PRK. On the other hand, wound healing complications are usually associated with PRK than LASIK because PRK involves the removal of the topmost layer of the cornea (epithelium). PRK can cause early complications such as ocular pain and delayed re-epithelialization and late complications such as corneal haze or scarring and refractive regression [Netto et al. 2005].

Several therapeutic measures are given to minimize complications following laser refractive surgery. Artificial tears, topical medications and occlusion of the tear duct are utilized for postoperative dry eye [Quinto et al. 2008]. While intraoperative and postoperative topical medications are given to modulate corneal wound healing [Dupps and Wilson 2006].

Lacritin is a lacrimal gland-secreted tear protein that has several properties which may be beneficial to those who undergo refractive surgery. It has been shown to promote lacrimal acinar cell secretion, stimulate corneal epithelial cell proliferation, and promote tearing [Sanghi et al. 2001, Wang 2006, Ma 2006, Spitze 2006, Ma 2008, McKown 2009]. Lacritin is also naturally bactericidal for both gram negative and gram-positive bacteria at low micromolar levels [McKown, ARVO 2009]. Previous studies showed lacritin stimulates regeneration of human corneal epithelial cells in vitro which may promote corneal wound healing [Sharma et al. ARVO 2009]. It has also been shown to stimulate tear production in previous animal studies [Spitze et al. 2006].

This study pursues the lacritin and its regulator heparanase response to the surgical stress of LASIK and PRK. Findings of this investigation may help direct future studies on recombinant lacritin to improve wound healing, visual outcomes, and dry eye following laser refractive surgery.

This is a collaborative study of the Warfighter Refractive Surgery and Research Center at Fort Belvoir (WRSRC) previously known as Center for Refractive Surgery at Walter Reed Army Medical Center, James Madison University (JMU) and the Rappaport Faculty of Medicine (RFM).

BODY

The present study specifically aims to characterize the response of human lacritin to the surgical

stress of PRK and LASIK. Lacritin has several properties that make it a very intriguing and potentially potent therapeutic adjunct in the modulation of post-refractive surgery wound healing. Firstly, it stimulates regeneration of human corneal epithelial cells in vitro and therefore may promote re-epithelialization following PRK and foster a more controlled stromal wound healing process with the potential to improve the accuracy of refractive outcomes.

Another consideration for lacritin in refractive surgery is its potential role in the prevention or treatment of dry eye, which remains one of the most common reasons for patient dissatisfaction following LASIK. Based on its proven ability to stimulate tear production, we hypothesize that treatment with recombinant lacritin may ameliorate or prevent dry eye following laser refractive surgery. Before such targeted molecular therapy can be tested however, the normal lacritin response must be characterized.

Task 1: Laboratory and Regulatory Preparation

A) Complete development and validation testing of lacritin and HPSE assays

B) Obtain required approval from all applicable Investigational Review Boards

- **Task 1 A. Development and validation testing of lacritin assay.**

  Lacritin Peptide, Anti-N-Terminal Anti-lacritin (Pep Lac N-Term) polyclonal antibodies were generated in rabbits against a synthetic peptide corresponding to the first 19 N-terminus amino acids of mature human lacritin as previously described (Seifert et al. IOVS 2012). To assess and validate Anti-Pep Lac N-Term specificity, an Enzyme-Linked Immunosorbent Assay (ELISA) was developed and tested. Specificity was also validated by Western Blot Analysis and is shown in Figure 1.

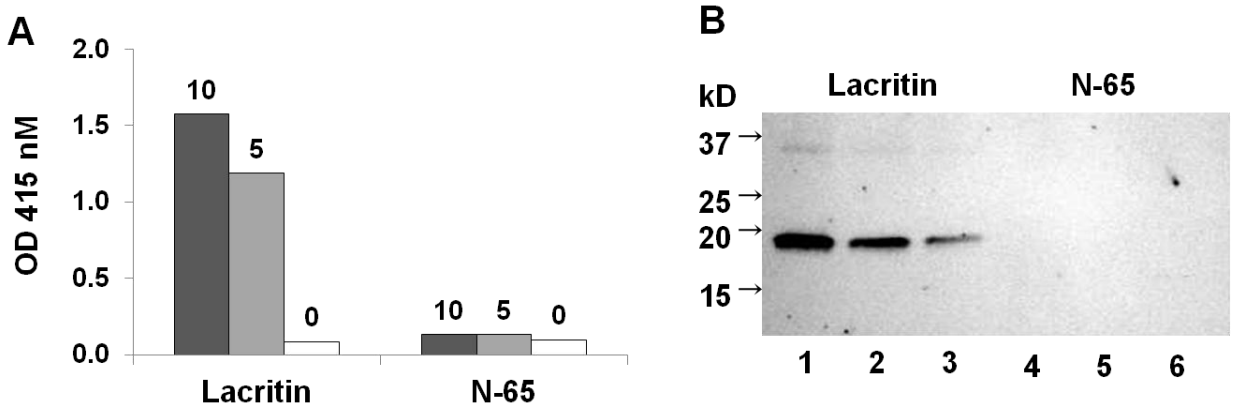

Figure 2. Specificity Anti-Pep Lac N-Term antibodies for full length lacritin, and lack of reactivity with N-65. (A) ELISA incubated with Anti-Pep Lac N-Term antibodies against 0, 5, and 10 ng of lacritin or N-65. (B) Western blot of decreasing amounts of purified lacritin and N-65 incubated with Anti-Pep Lac N-Term antibodies and developed via chemiluminescence: lanes 1 and 3, 400 ng; lanes 2 and 5, 200 ng; lanes 3 and 6, 100 ng.

An ELISA protocol was developed in which plates were coated overnight with 100 μL of lacritin or a delection mutant of lacritin lacking the first 65 amino acids (N-65) diluted 0, 50, or 100 ng/mL in coating buffer (0.017 M $NaHCO_3$, 0.015 M Na_2CO_3, pH 9.6). When assaying tear samples, 100 ng of total tear protein was coated in each well. To generate a standard curve of recombinant lacritin, each plate contained triplicate wells to which was added 0, 2, 4, 6, 8, 10, 12, 14, or 16 ng of recombinant lacritin protein. Wells were washed, blocked with PBS-Tween (PBS with 0.3% Tween-20 (PBS-T)), and then incubated for 1 h at 37°C with 100 μL of Anti-Pep Lac N-Term antiserum or preimmune serum diluted 1:200 in PBS-T. After washing 3 times with PBS-T, HRP-conjugated goat antirabbit IgG (MP Biomedicals, Solon, OH) diluted 1:1,000 in PBS-T was added for 1 h (37°C). Plates were washed 3 times with PBS-T, and then bound antibody was measured after incubation for 10 min with 100 μL of OPD substrate (Acros Organics, Geel, Belgium) by absorbance at 415 nm (Model 680, Bio-Rad, Hercules, CA). The same ELISA protocol was used for determining human tear lysozyme concentrations with lysozyme from human milk (Sigma-Aldrich, St. Louis, MO) for the standard curve and rabbit antihuman lysozyme polyclonal antibodies (MP Biomedicals, Solon, OH) diluted 1:200 in PBS-T for detection. For statistical significance, tear samples were analyzed in triplicate with duplicate plates The lacritin ELISA assay was validated with tears from healty adults as previously reported (Seifert et al. IOVS 2012).

- **Task 1 A Development and validation testing of HPSE assay.**
- As described in the research proposal, following the elution of tear samples at JMU, one half of the eluted volume was shipped to the Cancer and Vascular Biology Research Center, Rappaport Facility of Medicine, Technion in Haffa Israel for testing of heparanase (HPSE). Although the HPSE assay was validated as measured by background, standard curve, and

positive and negative controls, the levels of HPSE in all samples was low, most even below the detection limit. In order to increase the detection limit, another shipment of tear samples was sent to Israel containing 90% of the eluted volume. Although HSPE was detected in these samples above background, the entire sample was used in a single well. Considering that the lacritin ELISA was done in triplicate with duplicate plates (a total of six wells per sample) to generate enough data for statistical significance and 90% of each sample was needed to generate a single data point, the HPSE assay could not be used with the sample volumes in this study.

- **Task 1 B** IRB approval was obtained from Walter Reed Army Medical Center's Department of Clinical Investigation (WRAMC DCI), now known as Walter Reed National Military Medical Center Department of Research Programs (WRNMMC DRP), in an approval letter dated 14 December 2009 and Clinical Investigation Regulatory Office (CIRO) approval on 27 January 2010. The JMU IRB approved the study on 28 November 2011. WRSRC HRPO approval was obtained 13 January 2012. JMU HRPO approval was obtained 8 March 2012. Continuing review approval from WRNMMC IRB was obtained 11 October 2013 and from JMU IRB November 2012.

  Research Administrative Updates:

  - For this review period, a modification was submitted as part of the 2012 continuing review report requesting removal of investigators no longer participating in the study (**Appendix 1)**.
  - The WRNMMC IRB approval for the 2012 continuing review report for this study was approved 11 October 2013. (Appendix 1)
  - The current JMU IRB approval ID number is 12-0146 and is attached as Appendix 1.

Task 2: Study Execution and Laboratory Analysis

A) Enroll, perform baseline testing, and treat all study subjects

B) Collect postoperative data and tear samples (through 6 months post-op) and ship tear samples to JMU

C) Perform lacritin and HPSE assays on all pre- and post-operative tear samples

- **Task 2 A and B** Screening and enrollment is complete in the PRK male group. We are still actively enrolling in the PRK female group and the LASIK male and female groups. Ocular health examination and tear sample collection are completed at the pre-operative examination and at one day, one week, one month, three month, and six month follow up examinations. **Table 1** summarizes the progress of enrollment and follow up rates by group as of September 2013.

Table 1. Summary of enrollment and follow up rates.

	Enrolled			**1M**		**3M**		**6M**		**12M**	
	PRK (M/F)	LASIK (M/F)		PRK (M/F)	LASIK (M/F)	PRK (M/F)	LASIK (M/F)	PRK (M/F)	LASIK (M/F)	PRK (M/F)	LASIK (M/F)
Total required	49/49	49/49	Seen for Visit	47/29	22/6	47/27	17/6	44/22	15/6	37/15	13/6
Withdrawn	3/2	2/1	Missed Visit	0	0	0	0	3/2	0	7/3	0
Enrolled	49/31	28/8	Total Eligible	47/29	22/6	47/27	17/6	47/24	15/6	44/18	13/6
				100%	100%	100%	100%	93.6% / 91.7%	100%	84.1% / 83.3%	100%

- There were no adverse events reported since the last continuing review October 2012 to September 2013.

- For PRK, 276 samples from male participants and 148 samples from female participants have been shipped to JMU for analysis. For LASIK, 90 samples from male and 36 samples from female participants have been shipped to JMU for analysis.

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- **Task 2 C** A total of 360 tear samples have been received and processed to date at JMU. Upon receipt, samples are stored at -70 degrees C until processed for analysis. Tear samples are eluted from the collection wicks and assessed for total protein concentration by the BCA assay (Thermo Scientific BCA Protein Assay Kit; Pierce Biotechnology, Rockford, IL.). Samples are normalized to 100 ng total protein per well and assayed for lacritin content with the ELISA in

duplicate plates with triplicate wells for each plate. **Table 2** one summarizes the samples assayed to date. P denotes tear samples from PRK patients and L denotes LASIK patients.

Table 2. Summary of Tear Samples Analyzed at JMU

ELISA	Received	Assayed	Samples	Patients
P01-P49 (M)	P01-P33	P01-P33	180	33
P50-P98 (F)	P50-P65	P50-P65	84	16
L01-L49 (M)	L01-L12	L01-L12	66	12
L50-L98 (F)	L50-L54	L50-L54	30	5
Total Patients				66

Task 3: Data Analysis and Reporting

A) Periodic (quarterly) project review and interim data analysis

B) Final analysis of data, preparation of MS for publication

C) Final report to TATRC/MRMC

- **Task 3 A-1** Interim Data analysis: visual outcomes of participants who underwent PRK (66 participants;132 eyes) and LASIK (20 participants; 40 eyes) seen at 6 months postoperatively are summarized in Figures A-F.

Figure A. Uncorrected Distance Visual Acuity preoperatively and six months postoperatively

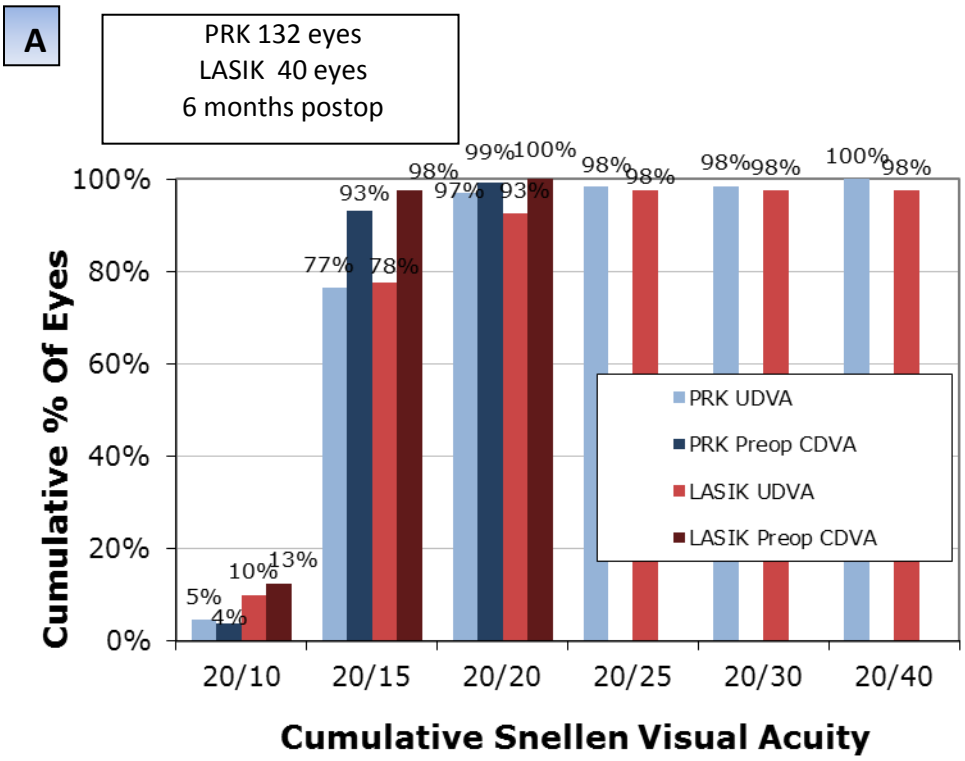

A
PRK 132 eyes
LASIK 40 eyes
6 months postop
Cumulative % Of Eyes
100%
80%
60%
40%
20%
0%
5%
4%
10%
13%
77%
93%
78%
98%
97%
99%
93%
100%
98%
98%
98%
98%
100%
98%
PRK UDVA
PRK Preop CDVA
LASIK UDVA
LASIK Preop CDVA
20/10
20/15
20/20
20/25
20/30
20/40
Cumulative Snellen Visual Acuity

Figure B. PRK vs. LASIK Spherical equivalent refractive accuracy six months postop.

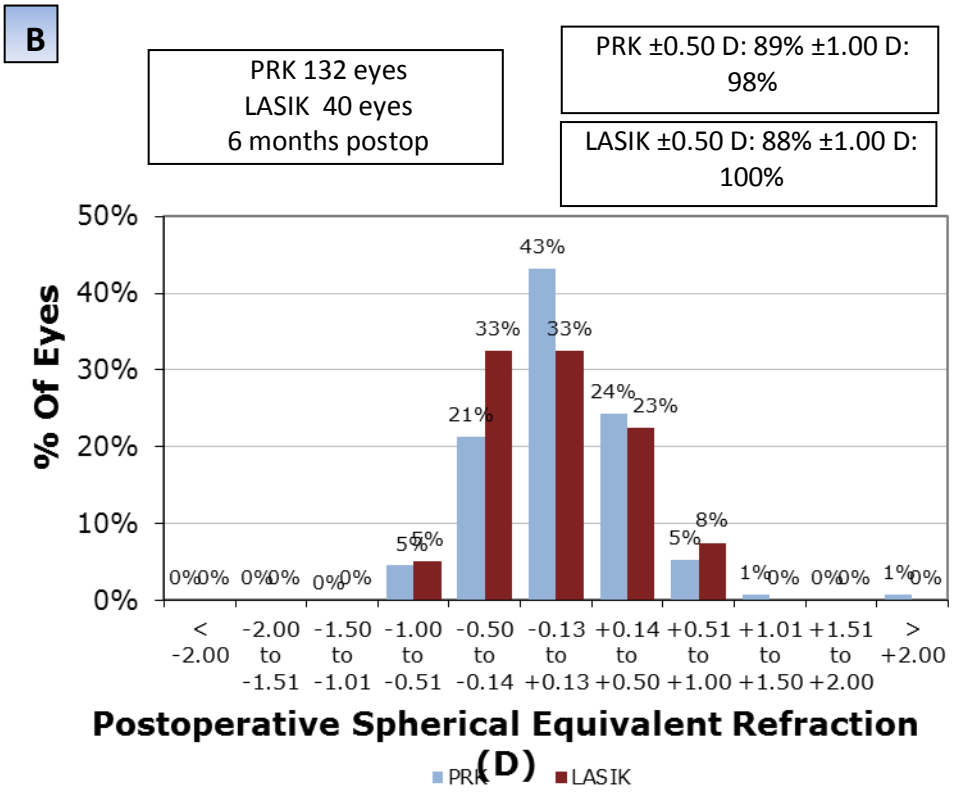


Figure C. PRK efficacy: attempted vs. achieved at six months postop.

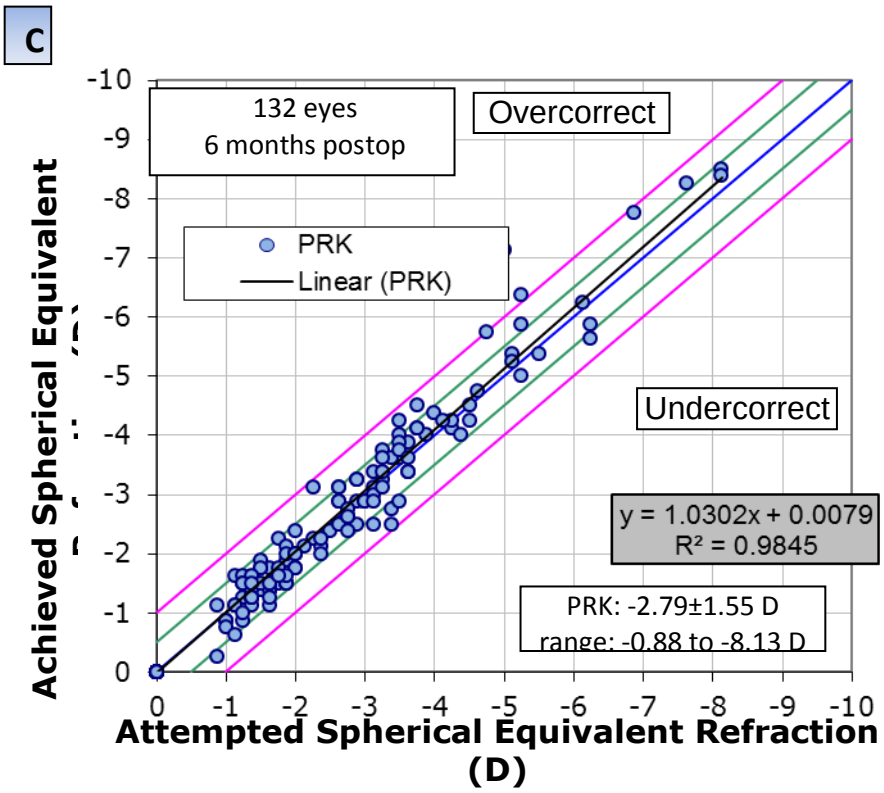


Figure D. LASIK efficacy: attempted vs. achieved at six months postop.

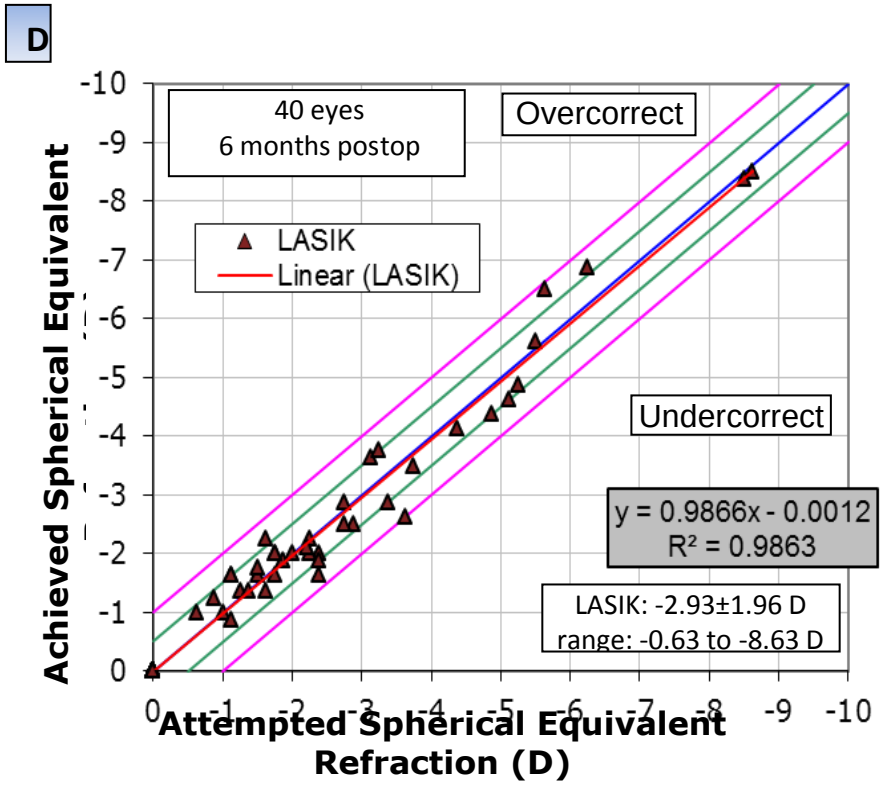


Figure E. Stability of spherical equivalent refraction.

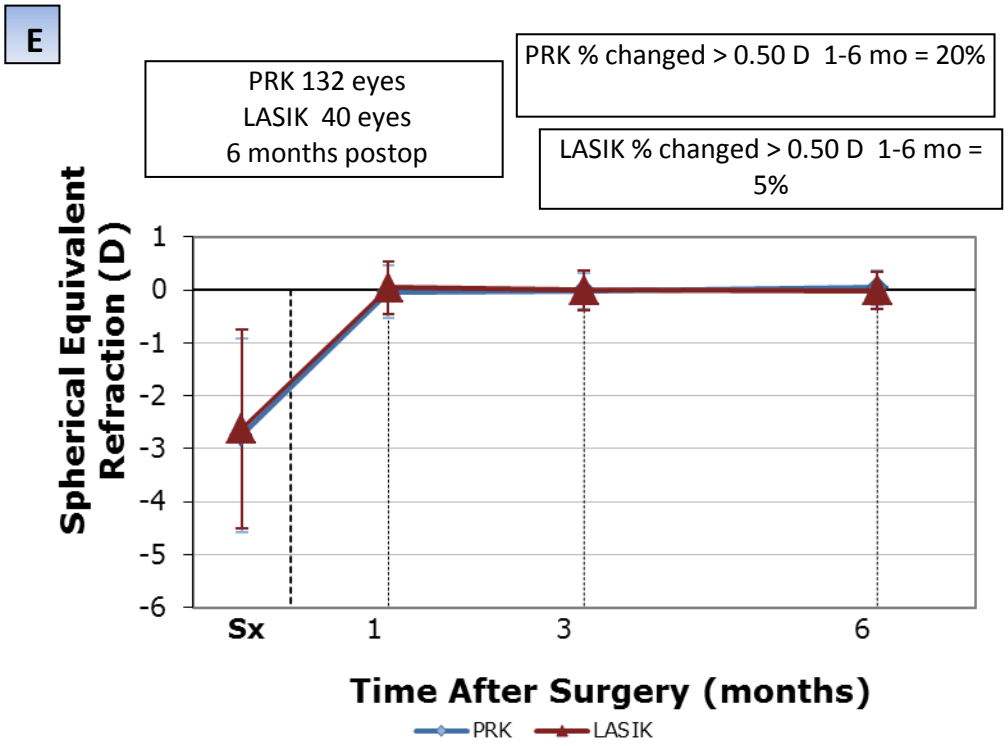
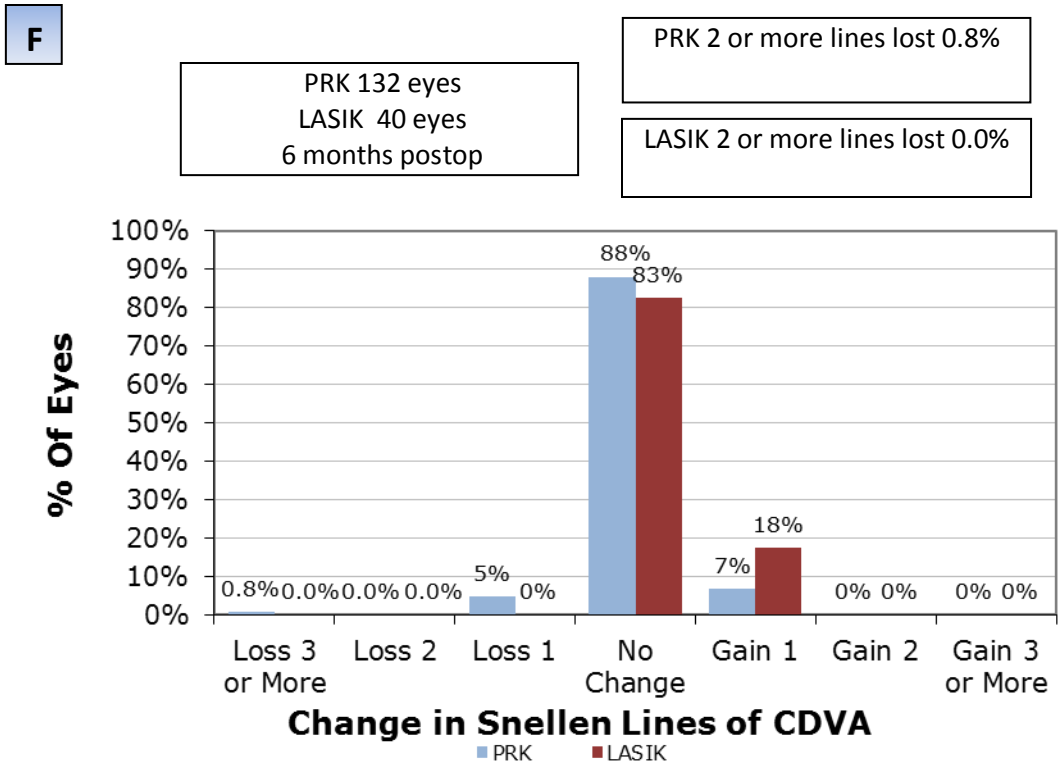


Figure F. Change in Corrected Distance Visual Acuity.



- **Task 3A-2** Two abstracts were submitted and are pending acceptance to the American Society of Cataract and Refractive Surgery Annual Meeting 2014:
    - A preliminary analysis of 52 participants was conducted to determine whether tear protein lacritin affects optical quality following PRK. Visual acuity, manifest refraction and TMS-4 (Tomey) surface regularity index (SRI), surface asymmetry index (SAI), and irregular astigmatism index (IAI) were determined preoperatively and postoperatively. Lacritin concentrations from tears collected pre- and postoperatively were determined using enzyme linked immunosorbent assay (ELISA). Multivariate analysis of variance was performed to determine if lacritin had any significant effect on optical quality indices after PRK. Mean manifest spherical equivalent was -2.86±1.70 diopters and mean ablation depth was 46.2±21.6μm. Lacritin concentration decreased significantly from preop (12.1±2.6 average %) at day 1 postop (10.4±4.4 ave. %, $p=0.03$) then increased significantly from day 1 to week 1 postop (12.7±2.6 ave. %, $p<0.01$). Ablation depth was associated with lacritin concentration at 1M postop (B coefficient = 0.23, $p=0.04$). Postoperative SAI and IAI changed significantly over time ($p<0.01$) while SRI did not ($p=0.76$). Lacritin did not have any significant affect on TMS indices at preop ($p=0.20$) or at 1M ($p=0.70$) and 3M postop ($p=0.78$). Preliminary results showed lacritin levels did not affect optical quality, as measured by TMS indices.
    - An interim analysis including 52 participants was conducted to determine whether levels of tear protein lacritin correlate with signs and symptoms of dry eye after PRK. Schirmer tear test scores, tear break up time (TBUT), corneal surface staining and dry eye symptoms were determined preoperatively and postoperatively. The Ocular Surface Disease Index (OSDI) questionnaire was used to evaluate dry eye symptoms. Lacritin concentrations from tears collected pre- and postoperatively were determined using ELISA. Multivariate analysis of variance was performed to determine if lacritin had any significance in signs and symptoms of dry eye. Mean participant age was 30.5±6.7 years, 65.4% of which were male. OSDI scores were significantly higher compared to preop (6.5±10.4) at 1 month (18.3±13.4, $p<0.01$) and 3 months postoperatively (11.5±8.4, $p=0.02$). Postoperative schrimer scores, TBUT, and staining scores were not significantly different from their preoperative values. Lacritin did not significantly affect dry eye markers at preop ($p=0.49$), at 1M ($p=0.44$), and 3M postop ($p=0.08$). Initial results suggest no relationship between tear lacritin and dry eye clinical indicators up to 3 months post-PRK.

- **Task 3A Lacritin tear sample analysis**

  Table 3 shows a summary of Tear Protein Concentrations for PRK and LASIK samples analyzed.

Table 3. Lacritin tear protein concentrations

	Tear Total Protein Concentrations (µg/mL)				
	PREOP	1 DAY	1 WK	1 MO	3 MO
P09	1612	636	397	1004	141
P10	1994	723	483	184	628
P11	2087	668	1311	850	154
P12	968	558	787	608	346
P13	1505	930	1203	806	775
P14	2211	1436	2499	1355	1091
P15	544	1038	343	624	1493
P16	2378	1032	2050	3355	1782
P17	650	522	1257	388	611
P18	1487	982	819	637	986
P19	1131	695	2109	1150	1322
P20	320	500	2173	1266	1505
P21	577	266	856	159	722
P22	652	636	548	1362	936
P23	879	573	306	984	606
P24	1547	490	1460	1872	826
P25	730	978	792	598	833
P26	1361	834	962	1235	565
P27	1286	1241	776	1502	815
P28	2199	569	2214	1622	1061
P29	389	834	1409	711	391
P30	2458	874	1088	1144	842
P31	1345	1386	864	560	763
P33	1712	439	2089	949	2000
P34	902	1083	769	740	405
P35	1117	741	880	505	893
P38	1965	442	1507	1327	1051
P39	949	287	1170	1481	1841
P41	1645	292	1516	1802	1866

	PREOP	1 DAY	1 WK	1 MO	3 MO
P42	1003	559	2138	1446	1344
P43	317	439	544	566	460
P44	1008	514	746	744	863
P46	1188	370	801	1068	1083
P47	2020	414	1717	712	1333
P49	512	532	797	767	297

P50	1368	1008	889	494	944
P51	1055	770	1665	869	1301
P55	1654	782	768	1335	814
P56	1944	917	1751	1740	1811
P57	1089	760	1502	1028	687
P58	2511	1080	2890	1757	1246
P59	1095	1297	3165	398	2360
P61	2337	1011	2616	1473	472
P62	294	142	380	204	386
P64	320	1202	768	1012	657
P65	860	227	1557	752	789
P67	623	499	731	1094	742
P68	1220	591	1459	1309	891
P69	1565	371	1246	2279	3135
P73	794	484	990	501	528
P74	1016	337	1607	1770	1107
L01	1057	761	485	613	458
L04	1219	1823	2300	2044	1462
L05	917	653	995	2197	1604
L06	2026	1586	938	662	545
L07	1251	781	1195	1691	673
LO8	1000	925	795	792	895
L10	1697	1439	1247	876	739
L11	1984	1243	833	1503	820
L12	1296	1386	1719	1380	630
L13	99	209	327	287	225
L14	912	816	604	558	718
L15	833	484	571	917	920
L50	758	549	277	987	965
L51	1468	1257	1986	1017	1384
L52	2202	1382	1307	1476	226
L53	2050	763	919	1397	545
L54	1078	837	448	1271	1241
L55	268	196	180	166	180

Summary of ELISA Lacritin Data for PRK and LASIK Samples

Table 4 shows a summary of all PRK samples assayed for lacritin for each time point and **Figure G** is a graph of average % lacritin for all PRK tear samples (% Lacritin = ng Lacritin/100 ng Total Protein) and **Figure H** shows the graph of average % lacritin for 16 full stes of all time points for PRK tear samples. **Figure I** is a scatter plot of the distribution and

average % lacritin for full stes of all time points for PRK tear samples and **Figure J** is a scatter plot of the distribution and average % lacritin for 10 full stes of all time points for LASIK tear samples. **Figure K** shows a preliminary analysis of tear lacritin concentration (average % lacritin) preop and up to 3 months postoperatively from 53 PRK participants and 18 LASIK participants.

Table 4. PRK Samples

Time	Samples
Preop	41
1 Day	39
1 Week	38
1 Month	40
3 Month	40
6 Month	19

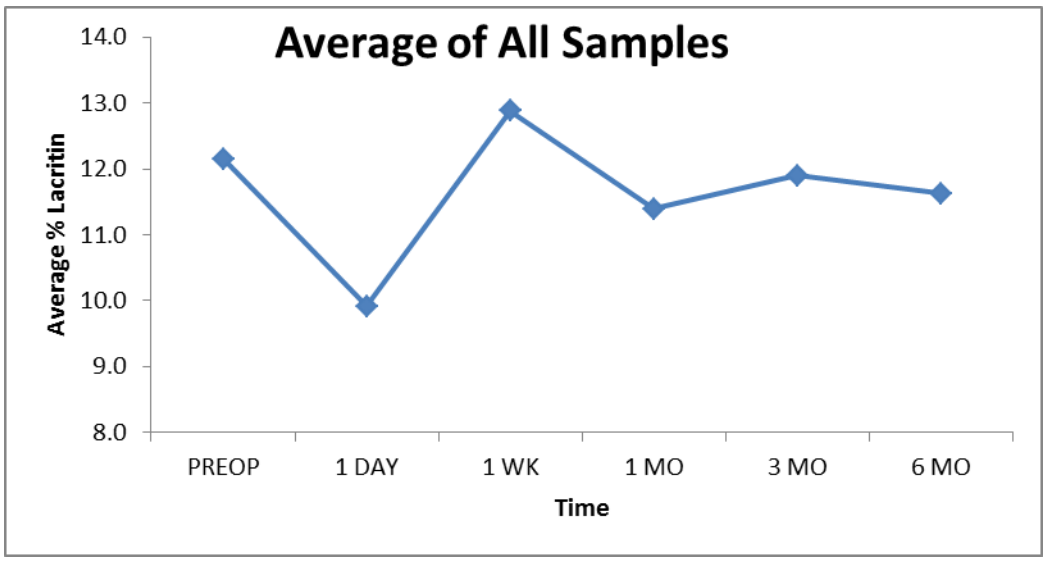


Figure G. Average % lacritin for all PRK tear samples (% Lacritin = ng Lacritin/100 ng Total Protein).

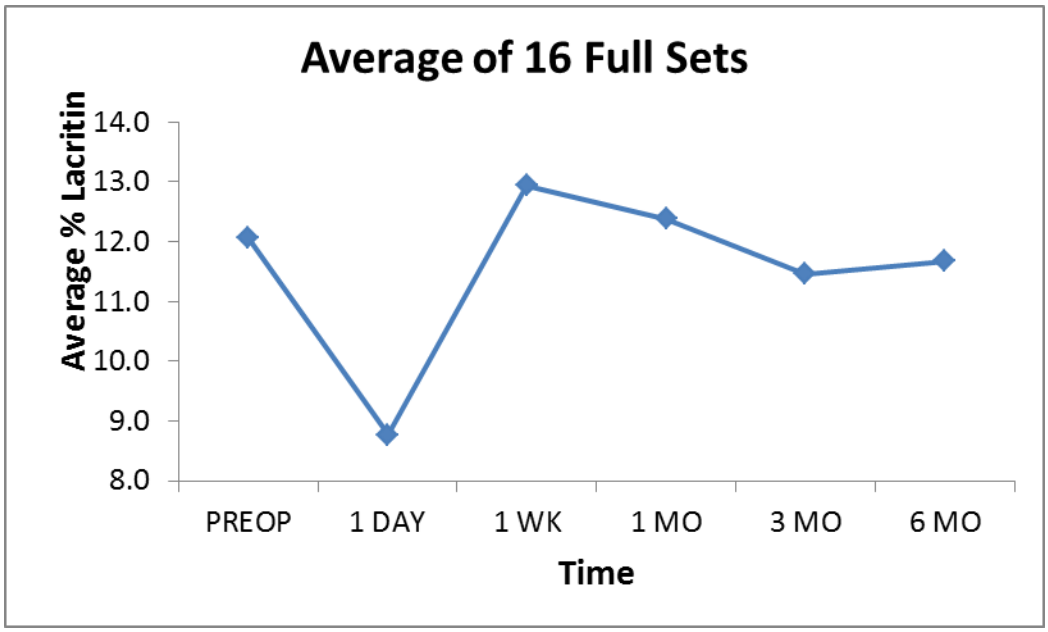


Figure H. Average % lacritin for full stes of all time points for PRK tear samples (% Lacritin = ng Lacritin/100 ng Total Protein).

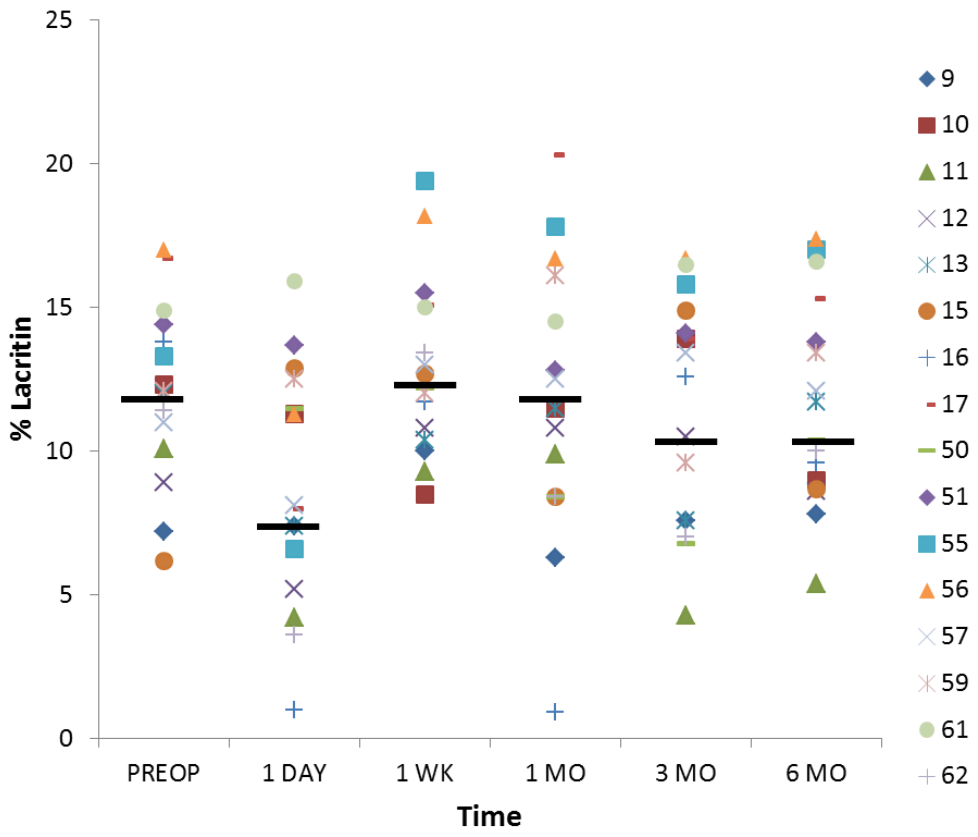


Figure I. Distribution and average % lacritin for full stes of all time points for PRK tear samples (% Lacritin = ng Lacritin/100 ng Total Protein).

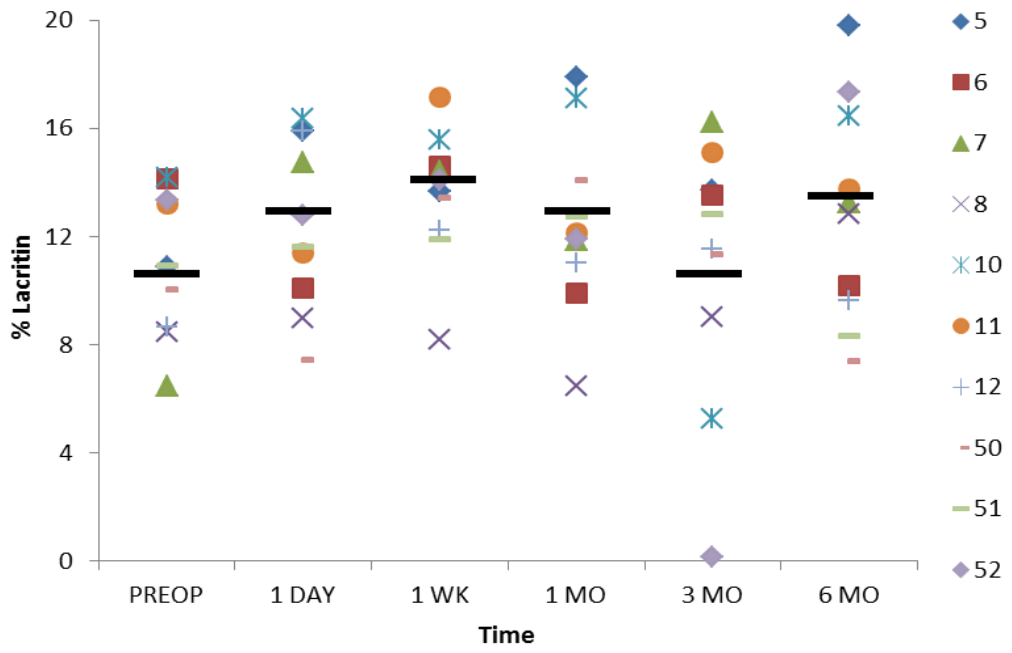
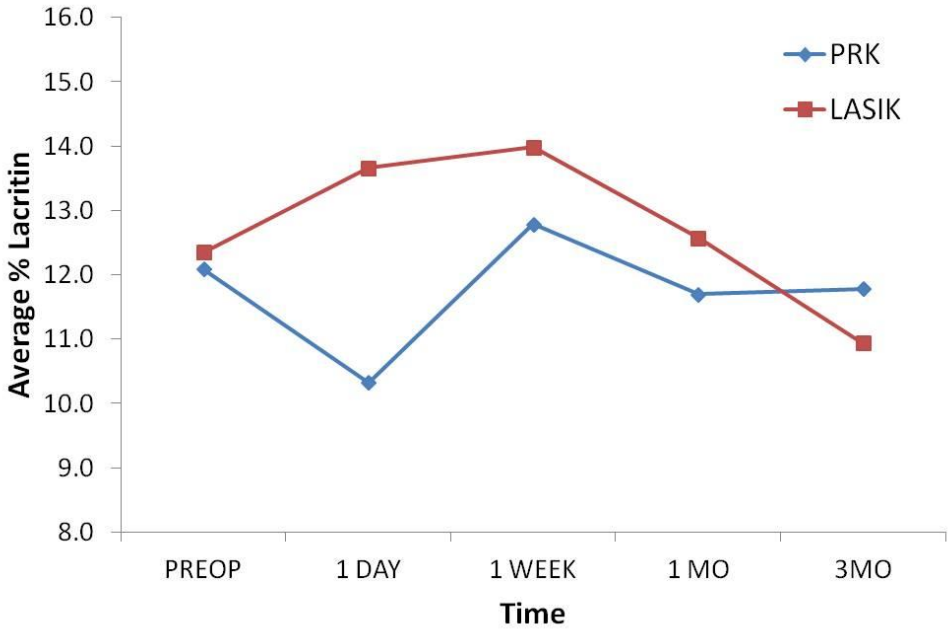


Figure J. Distribution and average % lacritin for 10 full stes of all time points for LASIK tear samples (% Lacritin = ng Lacritin/100 ng Total Protein).

Figure K. Average % lacritin concentration before and after PRK and LASIK.



- **Task 3 B and C** Due to the delay in opening the FBCH WRSRC caused by unanticipated operating suite environmental issues, a no-cost extension request was submitted 23 May 2013 to extend the program through 27 October 2014. This extension was approve 23 October 2013.

KEY RESEARCH ACCOMPLISHMENTS

- Interim analysis suggests lacritin levels did not affect optical quality of participants who underwent PRK, as measured by TMS indices up to three months postoperatively.
- Prelimnary analysis suggests no relationship between lacritin and dry eye clinical indicators up to three months post-PRK.

REPORTABLE OUTCOMES

None.

CONCLUSION

The objective for this study was to characterize the response of lacritin in participants undergoing LASIK and PRK. Preliminary data in PRK participants shows there is a significant difference in lacritin levels within in the early postoperative period and between surgical procedures. It is unknown whether such differences would have a meaningful impact on visual outcomes or optical quality. Based on the PRK study results to date, there is no correlation. Ongoing testing in this study will help determine if there is a difference in lacritin levels in participants undergoing LASIK as well as if lacritin concentrations affect early and long-term optical quality and visual outcomes in PRK and LASIK participants.

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SUPPORTING DATA

None

APPENDICES

Appendix 1 - WRNMMC IRB approval and acknowledgement letters, current consent form, and JMU IRB approval.

Appendix 2- Lacritin tear sample raw data.

WALTER REED NATIONAL MILITARY MEDICAL CENTER
INSTITUTIONAL REVIEW BOARD

8901 WISCONSIN AVENUE
BETHESDA MARYLAND 20889-5600

Date: October 11, 2013

From: WRNMMC IRB
To: Denise Ryan

Subj: WRNMMC IRB REVIEW OF 351515-20

PROJECT TITLE:	Lacritin and Heparanase Levels in Human Tears after Laser Refractive Surgery
REFERENCE #:	351515-20
SUBMISSION TYPE:	Continuing Review/Progress Report
ACTION:	APPROVED
APPROVAL DATE:	October 10, 2013
EXPIRATION DATE:	October 19, 2014
REVIEW TYPE:	Full Committee Review

1. The IRB reviewed your continuing review report and amendment at their meeting on October 10, 2013. Your Minimal Risk protocol continues to meet the requirements under 32 CFR 219.111. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All future research must be conducted in accordance with this approved submission

2. The IRB notes the presence of a Research Monitor on the study team. Please note that the IRB directs the research team to maintain a Research Monitor if they wish, but this role is not required for minimal risk studies. If the decision is made to no longer utilize the role of the Research Monitor, please submit an amendment with all study documents needing revision.

3. The following documents have been updated with this submission:

- Protocol- Version 6, 23 August 2013
- Consent Form- Version 4 September 2013
- DMRN Research Project Coversheet- Version 3 September 2013

4. The IRB approved, stamped consent/HIPAA authorization form is to be duplicated and used to enroll subjects at **Fort Belvoir Community Hospital (FBCH)**. Keep the signed, original consent forms in your project file; give each subject a signed copy of the consent form.

5. You are reminded to provide all amendments, deviations, related serious adverse events, unanticipated problems involving risks to subjects or others, and any other pertinent information regarding this research protocol to the Department of Research Programs through IRBNet for reporting to the IRB.

6. You are reminded that all presentations and publications related to this work must cleared through the publications clearance process.

7. If you have any questions, the POC is Debarati Dasgupta at 301 400-0692 or debarati.dasgupta.civ@health.mil. Please include your project title and reference number in all correspondence with this committee.

This document has been electronically signed in accordance with all applicable regulations, and a copy is retained within our records.

04 September 2013

FORT BELVOIR COMMUNITY HOSPITAL (FBCH)
FORT BELVOIR, VA

This Clinical Trial consent form is valid only if it contains the IRB stamped date.

Consent for Voluntary Participation in a Clinical Trial (a type of research study) Entitled: "Lacritin and heparanase levels in human tears after laser refractive surgery"

Principal Investigator: Denise S. Ryan, Ophthalmology Service, Department of Surgery (571) 231-1600

Study Sites :XX FBCH

Standard of Care (SOC) and Recruitment Site: XX WRNMMC (Walter Reed National Military Medical Center)

1. INTRODUCTION OF THE STUDY

You are being asked to participate in this study because you are an active duty U.S. military personnel and have elected to undergo either photorefractive keratectomy (PRK) or laser-assisted in situ keratomileusis (LASIK) eye surgery to correct your vision. Your participation is entirely voluntary. Refusal to participate will not result in any penalty or loss of benefits to which you are otherwise entitled, nor will your refusal affect your employment or career status.

2. PURPOSE OF THE STUDY

Although over a million laser refractive procedures are performed each year, differences in wound healing continue to cause unpredictability in outcomes and in some cases lead to complications. The human tear protein lacritin has been shown to contribute to wound healing and may improve dry eye. Lacritin activity is regulated by the enzyme heparanase (HPSE) that acts as an on/off switch for lacritin. The purpose of this study is to measure levels of lacritin and HPSE in tears of patients undergoing PRK and LASIK. Better understanding of the lacritin and HPSE response to laser refractive surgery will potentially lead to advances in wound healing and may prevent or reduce dry eye.

Other studies have shown PRK and LASIK surgery to be safe and effective in the treatment of nearsightedness, farsightedness and astigmatism (unequal curvature of the eyeball) in civilians and U.S. personnel. However, dry eye is a complication that can cause considerable problems in a small number of patients after otherwise successful surgery. Tears play a great role in eye health; they are a complex fluid containing many different compounds created in different glands and cells. Because of the many origins of tear components, it is often difficult to determine which component, if any, is involved in eye disease. To determine lacritin and HPSE's changes

after refractive surgery, we will collect and test samples before and after PRK and LASIK surgery.

The tear collection process we will be using employs a polyester fiber rod which has been shown to be a quick, non-invasive method of collecting tears.

3. PROCEDURES TO BE FOLLOWED

If you agree to be in this study, you will undergo either PRK or LASIK surgery on both of your eyes. Which surgery you have will be determined by you and your doctor. Your surgery will be done the same way as it would be done if you were not taking part in this study. You will have comprehensive eye examinations done prior to the laser surgery, 1, 3 (PRK only), and 7 days immediately after the procedure, and at 1, 3, 6, and 12 month visits postoperatively (after surgery). These appointments are "standard of care"- in other words, you would be asked to come to the clinic for these visits even if you were not taking part in this study. Information needed for your surgery and postoperative care, which is considered to be the standard of care, will be recorded during these visits for research purposes. This will include information about how well you see, and your refraction (the need for glasses), eye pressure, corneal (the clear transparent outer layer of the eye) curvature, corneal clarity, and corneal thickness.

Several eye examinations will be done specifically due to your participation in this study and are therefore being done for research purposes. These additional tests will occur at the standard visits before surgery and at the examinations done at the 1, 3, 6, and 12 months after surgery and will add an additional 30 minutes to your examination time. In addition, tear sampling will also be done at the 1 day and 1 week post-operative visit. Each of these tests has been used in clinical practice for years. They are being done for research purposes in this study so that we can attempt to find a relationship between the tear lacritin and HPSE levels, results of these tests, and any symptoms of dry eye you might experience.

The following are the additional tests at each visit:

1. Questionnaire: At each of these examinations you will also be asked to complete a questionnaire (will take about 5 minutes) for research purposes about any dry eye symptoms you may be experiencing and things that may cause you eye irritation. You will complete this questionnaire before surgery and at the 1, 3, 6 and 12 month visits after surgery.
2. Computerized corneal mapping: we will perform computerized corneal mapping to detect changes on the surface of your eye. During this test you will be seated in front of a machine that takes a picture of your eye and uses a computer program to analyze the picture. The machine doesn't touch your eye, is operated by a fully trained technician, and takes less than five minutes to complete. This will be done on both eyes before surgery and at the 1, 3, 6 and 12 month visits after surgery.
3. Tear Collection: At the eye examination done before surgery, you will be asked to

undergo a tear collection procedure. If you are wearing contact lenses, you must remove your lenses and wait 5 minutes before proceeding with the tear collection procedure. To collect your tears, a drop of 0.5% proparacaine, a local anesthetic, will be placed in the left eye. You will wait with your eyes closed for two minutes. A small polyester fiber rod will be placed in contact with the tear fluid at the corner of your eye to extract the tears for 3-5 minutes. The tear collection procedure will not hurt but may be uncomfortable. This process will be repeated on your left eye at the 1 day, 1 week, 1, 3, and 6 month post-operative appointments.
The collected tears will be sent to James Madison University (JMU) in Harrisonburg, Virginia for tear separation. Each tear sample will be split in half: the first half of each sample will be tested at JMU and the remaining half will be sent to the Rappaport Faculty of Medicine (RFM) in Haifa, Israel. These analyses will determine the profile of lacritin and HPSE in tears in response to laser refractive surgery. You do not need to take any precautions or actions prior to the collection of the tears. No personal identifying information will be sent with your tear samples to JMU or RFM. Your samples will be labeled with only a study ID number, gender and age and will not contain any part of your name or social security number. The tears collected will be destroyed in the analysis process, thus no tears are retained after completion of the assay.

4. Lissamine green stain: The doctor will examine the surface of your eye after a dye has been put in it. This test will be performed on both eyes before surgery and at the 1, 3, 6 and 12 month visits after surgery.
5. Schirmer test: This is a measurement of the amount of your tear production. You will be given an anesthetic drop (proparacaine), asked to wait 2 minutes and then fixate on an object with a slightly upward gaze and minimal blinking while a small test strip is placed on your lower eyelid. You can either keep your eyes closed gently or maintain an upward gaze with minimal blinking for 5 minutes. This test will be performed on both eyes before surgery and at the 1, 3, 6 and 12 month visits after surgery.
6. Tear break up time: We measure the time required for a dry spot to appear on your corneal surface after blinking. For this test, the surface of your eye will be touched with a small test strip containing a dye. You will be asked to blink several times to distribute the dye over your eye surface and then stare straight ahead without blinking while the doctor looks at your eye with a special light and checks how long it takes for a dry area to appear on your corneal surface. This test will be repeated five times each time it is done. This test will be performed on both eyes before surgery and at the 1, 3, 6 and 12 month visits after surgery.

The FBCH Clinic can be contacted at (571) 231-1600 and the WRNMMC clinic can be reached at (301) 295-1339.

4. AMOUNT OF TIME FOR YOU TO COMPLETE THIS STUDY

You will be part of this study for a total of one year. During this time, you will not be asked to make any extra visits to the clinic for the purposes of this study. All of the information and

procedures needed for this study will be done at standard of care visits. The eye examination done before surgery and the visits 1, 3, 6, and 12 months after surgery will take about 30 minutes longer than it would if you were not taking part in this study. The one day and 1 week visits after surgery will take 10 minutes longer than they would if you were not taking part in this study. The total amount of additional time required to participate in this study over the course of one year is approximately three hours.

5. NUMBER OF PEOPLE THAT WILL TAKE PART IN THIS STUDY

A total of up to 196 subjects are expected to take part in this study.

6. POSSIBLE RISKS OR DISCOMFORTS FROM BEING IN THIS STUDY

Any additional risks that may develop as a result of your participation in this study, other than those associated with the procedures themselves, are related to the tear collection. None of the testing procedures pose any risk beyond a normal eye examination. The following are possible risks or discomforts that may develop as a result of participation in this study:

The use of the anesthetic proparacaine may cause a mild stinging or eye irritation that may occur up to several minutes after the drop is applied. Burning, itching, pain, redness, swelling of the eye or eyelid, watering of the eyes or other irritation of eye, although rare, may also occur.

The tear collection procedure will not hurt, but may be uncomfortable. It is also possible, but very unlikely, that your eye could show a small amount of redness after the tear collection is completed. If this should happen, the redness should go away and no treatment should be needed. There may be excess tearing during the tear collection procedure. The surface of the eye could be accidentally scraped, but this would be highly unusual.

In addition to the above-mentioned risks, this study may involve risks to you that are currently unforeseeable.

While all possible risks that we know about have been listed above, other risks about which we do not know may occur or be discovered during future studies. If we find that there was a major risk to you that was not known at the time of your participation in the study, and the risk might have some effect on your health, you will be informed.

7. POSSIBLE BENEFITS FROM BEING IN THIS STUDY

You will not benefit from being in this study, but the information we learn may help us in determine how changes in lacritin and HPSE affect dry eye symptoms and wound healing after LASIK or PRK surgery.

8. CONFIDENTIALITY/PRIVACY OF YOUR IDENTITY AND YOUR RESEARCH RECORDS

The principal investigator will keep records of your being in this study. These records may be looked at by people from the Walter Reed Department of Research Programs (DRP), Fort Belvoir Community Hospital DRP, the Walter Reed Institutional Review Board (IRB), and other government agencies as part of their duties. These duties include making sure that research subjects are protected. Confidentiality of your records will be protected to the extent possible under existing regulations and laws. Complete confidentiality cannot be promised, particularly for military personnel, because information bearing on your health may be required to be reported to appropriate medical or command authorities. Your name will not appear in any published paper or presentation related to this study.

A folder will be maintained containing your study records. It will include a copy of this consent form, patient information sheets, your operative report and any other related correspondence. Patient data obtained during your eye examinations before and after surgery will be recorded on worksheets and will be maintained in the folder. To protect your confidentiality, your study records will be kept in a locked file cabinet by the study coordinator at Fort Belvoir Community Hospital, Ft. Belvoir, VA with access limited to the principal investigator, research director, technical staff and study personnel. When you enter this study, you will be assigned a study ID number which will not include any part of your name or social security number. A master list will be maintained that links your study ID number with your personal identifying information. The master list will be kept in a file separate from the patient records in a locked file cabinet at Fort Belvoir Community Hospital, Ft. Belvoir, VA. Samples sent to JMU or RFM will be labeled only with your study ID number, gender and age, and not any personal identifying information. Any data sent out for analysis will be de-identified (labeled without any of your personal identifying information).

9. CONDITIONS UNDER WHICH YOUR PARTICIPATION IN THIS STUDY MAY BE STOPPED WITHOUT YOUR CONSENT

Your taking part in this study may be stopped without your consent if remaining in the study might be dangerous or harmful to you. Your taking part in this study may also be stopped without your consent if the military mission requires it, or if you become ineligible for medical care at military hospitals. The principal investigator may terminate your participation in this study if you fail to attend the before or after surgery eye examinations or elect not to undergo the laser procedure.

10. ELIGIBILITY AND PAYMENT FOR BEING IN THIS STUDY

You will not receive any payment for being in this research study.

11. COMPENSATION IF INJURED AND LIMITS TO MEDICAL CARE

You will not receive any compensation (payment) should you be injured as a direct result of being in this study. You should understand that this is not a waiver or release of your legal rights. You should discuss this issue thoroughly with the principal investigator before you enroll in this study. Should you be injured as a result of your participation in this study, you will be given medical care for that injury at no cost to you.

Medical care is limited to the care normally allowed for Department of Defense health care beneficiaries (patients eligible for care at military hospitals and clinics). Necessary medical care does not include in-home care or nursing home care. If you need to be hospitalized, you may have to pay the normal fees for subsistence (hospital meals), as per standard regulations.

If at any time you believe you have suffered an injury or illness as a result of participating in this research project, and you are enrolled at WRNMMC you should contact the Department of Research Programs (DRP) at WRNMMC at 301-295-8239. If you are enrolled at FBCH you should contact Fort Belvoir Department of Research Programs at 571-231-4020.

12. COSTS THAT MAY RESULT FROM TAKING PART IN THIS STUDY

There is no charge to you for taking part in this study.

13. IF YOU DECIDE TO STOP TAKING PART IN THIS STUDY AND INSTRUCTIONS FOR STOPPING EARLY

You have the right to withdraw from this study at any time. If you decide to stop taking part in this study, you should tell the principal investigator as soon as possible; by leaving this study at any time, you in no way risk losing your right to medical care, nor will it affect your employment or career status. Some testing or period of observation by the investigators may be recommended for you in order for you to safely stop taking part in this study.

14. STEPS TAKEN BEFORE AND DURING THIS STUDY TO PROTECT YOU

To minimize the potential for increased irritation, you will be excluded from participation if you have an allergic reaction to 0.5% proparacaine or have been diagnosed with dry eyes or other surface conditions.

15. OTHER PROCEDURES OR TREATMENTS THAT YOU COULD CHOOSE

You may choose to have LASIK or PRK surgery without taking part in this study. You may also choose to have another refractive procedure done or to have a surgical alternative such as radial keratotomy or lens implants. Your doctor can provide you with more information about your

nearsightedness, farsightedness and astigmatism and the benefits and risks of the different treatments available. You are encouraged to discuss this with your doctor.

16. IMPORTANT NEW FINDINGS THAT MAY AFFECT YOUR WILLINGNESS TO STAY IN THE STUDY

If we learn new information during the study that could affect your decision to be in this study, we will tell you this information. For example, if we learn about new severe side effects of tear collection, we will tell you about these side effects. The results of the research will be provided to you if you so desire.

17. YOUR RIGHTS IF YOU TAKE PART IN THIS STUDY

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care.

18. AUTHORIZATION FOR RESEARCH USE OF PROTECTED HEALTH INFORMATION

The Federal Health Insurance Portability and Accountability Act (**HIPAA**) includes a Privacy Rule that gives special safeguards to Protected Health Information (**PHI**) that is identifiable, in other words, can be directly linked to you (for example, by your name, Social Security Number, birth date, etc.). We are required to advise you how your PHI will be used.

(1) What information will be collected?

For this research study, we will be collecting information about your eye examinations (including the SOC examination), refractive surgery, ocular (eye) health status, any side effects that you are experiencing, how the treatment affects your vision, and tear analysis.

(2) Who may use your PHI within the Military Healthcare System?

The members of the research team will have access to your health information in order to find out if you qualify to participate in this study, to plan your tear collection, and to analyze the research data. Additionally, your PHI may be made available to health oversight groups such as the Walter Reed Department of Research Programs, Fort Belvoir Community Hospital Department of Research Programs, the Walter Reed Institutional Review Board, and other government agencies as part of their duties.

(3) What persons outside of the Military Healthcare System who are under the HIPAA requirements will receive your PHI?

No one outside the Military Healthcare System will receive your PHI. Data and specimens sent to the James Madison University (JMU) in Harrisonburg, Virginia and the Rappaport Faculty of Medicine (RFM) in Haifa, Israel will be labeled only with your study ID number, age and gender and not any personal identifying information.

(4) What is the purpose for using or disclosing your PHI?

We will use your protected health information during the course of the research study to: monitor your health status, measure the effects of drugs/devices/procedures on you, gather samples, determine research results, and to possibly develop new tests and procedures. The information may also be reviewed when the research study is audited for compliance.

(5) How long will the researchers keep your PHI?

The study site research team will keep the research data and the master list linking your study ID number with your personal identifying information for up to seven years after the end of the study. At the end of this time the master list will be destroyed and the research data (without any information that can link it back to you) will be kept indefinitely.

(6) Can you review your own research information?

You have the right to view your personal research information at any time during the course of the study. When the study is over, you have the right to copy your research information for your records.

(7) Can you cancel this Authorization?

Yes. If you cancel this Authorization, you will no longer be included in the research study. However, the information that has already been collected will be kept by the research team to assure patient safety. If you want to cancel your Authorization, please contact the Principal Investigator in writing.

If you decide to participate in this research study, your Authorization for this study will not expire unless you revoke or cancel it in writing to the research doctor. If you revoke your Authorization, you will also be removed from the study, but standard medical care and any other benefit to which you are entitled will not be affected in any way. Revoking your Authorization only affects the use and disclosure (sharing) of information after your written request has been received.

(8) What will happen if you decide not to grant this Authorization?

If you decide not to grant this Authorization, you will not be able to participate in this research study. Refusal to grant this Authorization will not result in any loss of medical benefits to which you are otherwise entitled, nor will your refusal affect your employment or career status.

(9) Can your PHI be disclosed to parties not included in this Authorization who are not under the HIPAA requirements?

There is a potential that your research information will be shared with another party not listed in this Authorization in order to meet legal or regulatory requirements. Examples of persons who may access your PHI include representatives of the Army Clinical Investigation Regulatory Office, the Food and Drug Administration, the Department of Health and Human Services (DHHS) Office for Human Research Protections (OHRP), and the DHHS Office for Civil Rights. This disclosure is unlikely to occur, but in that case, your health information would no longer be protected by the HIPAA Privacy Rule.

(10) Who should you contact if you have any complaints?

If you believe your privacy rights have been violated, you may file a written complaint with (if you are enrolled at WRNMMC) the Walter Reed Privacy Officer, located at 8901 Wisconsin Avenue, Bethesda, MD 20889-5600, telephone 301-319-4775 or (if you are enrolled at FBCH) the FBCH Privacy Officer, FBCH Privacy Office, located at 9300 Dewitt Loop, Oaks Pavilion, Fort Belvoir, VA 22060 at 571-231-3319.

Your signature at the end of this document acknowledges that you authorize WRNMMC/ FBCH personnel to use and disclose your Protected Health Information (PHI) collected about you for research purposes as described above.

19. CONTACTS FOR QUESTIONS ABOUT THE STUDY

If you have questions about the study, or if you think you have a study-related injury you should contact Denise Ryan at 571-231-1600. For questions about your rights as a research participant, if you are enrolled at WRNMMC contact the Walter Reed Department of Research Programs at 301-295-8239 or the Walter Reed Staff Judge Advocate Office at 301-295-2215. If you are enrolled at FBCH, contact FBCH Clinical Investigations at 571-231-4020 or the Office of the Command Staff Judge Advocate in the Sunrise Pavilion at 571-231-2877.

A copy of this signed consent form and HIPAA authorization will be provided to you.

SIGNATURE OF RESEARCH SUBJECT

You have read the information in this consent form. You have been given a chance to ask

questions and all of your questions have been answered to your satisfaction.

BY SIGNING THIS CONSENT FORM, YOU FREELY AGREE TO TAKE PART IN THE RESEARCH IT DESCRIBES.

_____ _____

Subject's Signature Date

_____

Subject's Printed Name

SIGNATURE OF INVESTIGATOR

You have explained the research to the volunteer and answered all of his/her questions. You believe that the volunteer subject understands the information described in this document and freely consents to participate.

_____

Investigator's Signature Date (must be the same as the participant's)

_____

Investigator's Printed Name

Version – NCR Clinical trial protocol CF&HIPAA 3June2009.doc

JAMES MADISON UNIVERSITY.

SPONSORED PROGRAMS ADMINISTRATION

MEMORANDUM

TO: Dr. Kyle Seifert and Dr. Robert McKnown, Principal Investigators

FROM: Carolyn Strong, IRB Research Coordinator

DATE: November 7, 2012

RE: Human Research Protocol Approval

The Human Subject Research protocol entitled, "*Levels of the Novel Glycoprotein Lacritin in Human Tears After Laser Refractive Surgery*" has been approved by James Madison University's Institutional Review Board (IRB). A signed copy of the Action of the Board form is enclosed for your records. Your research protocol has been assigned the ID Number 12-0146.

As a condition of the IRB approval, your protocol is subject to annual review. Therefore, you are required to complete a follow-up report before your project end date. You *must* complete the follow-up report regardless of whether you intend to continue the project for another year. For your convenience, a hard copy is enclosed. An electronic copy of the follow-up report form can be found on the Sponsored Programs Administration web site at the following URL: http://www.jmu.edu/sponsprog/allforms.html#IRBform.

You are reminded that any changes in your protocol that affects human subjects must be submitted to the IRB for approval *before* implementing new procedures. This requirement applies to changes in subjects, equipment, procedures, investigators, survey tools, and location of the data collection site. Also, should any adverse events occur during your study, you are required to *immediately* notify Carolyn Strong, IRB Research Coordinator. To avoid confusion, please use the assigned protocol number when communicating with the IRB Research Coordinator about your project.

Federal Guidelines stipulate that you are required to keep a copy of your approved human subjects' protocol, including the approved informed consent form and site letter of permission, for at least three years after completion of your research. The protocol must be accessible for inspection and copying by authorized representatives of the department or agency supporting or conducting the research at reasonable times and in a reasonable manner. Please let me know if you need additional assistance or further clarification.

From the desk of...
Carolyn Strong, CIM, CRA
IRB Research Coordinator
Sponsored Programs Administration
James Madison University
JMAC Building 6, Suite 26, MSC 5728
Harrisonburg, VA 22807

strongcd@jmu.edu
Phone: 540-568-2318
Fax: 540-568-6240

JAMES MADISON UNIVERSITY

INSTITUTIONAL REVIEW BOARD

ACTION OF THE BOARD

Date: November 2, 2012 ID Number: 12-0146

Title of Study: *Levels of the Novel Glycoprotein Lacritin in Human Tears After Laser Refractive Surgery*

Principal Investigator(s): Dr. Kyle Seifert and Dr. Robert McKown

The Institutional Review Board took the following action on the human subjects study cited above:

__X__ Approved _____ Disapproved

Approval of the study is for the period from **11/2/2012** through **11/1/2013.**

The Investigator(s) shall immediately bring to the attention of the Institutional Review Board any changes proposed for the approved study as they relate to the care or use of human subjects. The IRB will decide whether the extent or type of changes proposed warrants formal committee review. If such a review is deemed necessary, the chairperson shall schedule the review for the earliest feasible time.

*FOR EXTERNALLY FUNDED PROJECTS, INVESTIGATOR(S) ARE RESPONSIBLE FOR CONVEYING A COPY OF THIS DOCUMENT TO THE OFFICE OF SPONSORED PROGRAMS TO BE FORWARDED TO THE APPROPRIATE FUNDING AGENCY.

David Cockley, Dr. PH (Chairperson) 11/05/12 Date

***Your Follow-up Report must be submitted within 30 days of the project end date listed above.**

***If you wish to continue your study past the approved project end date above, you must submit a Follow-up Report indicating an Extension Request, along with supporting information.*

Although the IRB office sends reminders, it is ultimately *your responsibility* to submit the continuing review report in a timely fashion to ensure there is no lapse in IRB approval.

Please return IRB Follow-up Report to the Office of Sponsored Programs: Campus Mail MSC 5728.

JAMES MADISON UNIVERSITY.

Follow-Up Report for Research Project

The Institutional Review Board (IRB) on the
Use of Human Subjects in Research
James Madison University

Protocol ID #:	
Title of Research:	
Name of Investigator(s):	
Signature:	**Date:**
Signature:	**Date:**
Signature:	**Date:**
Signature:	**Date:**

Faculty Advisor *(where applicable)*:	
Signature:	**Date:**

PROTOCOL STATUS: *(Please select all that apply and provide details as required.)*

☐ **Research was not conducted.**
(Please provide brief explanation.)

☐ **Research is now completed and was conducted according to the proposal submitted.**
(Please provide an abstract of findings or summary of progress to date.)

☐ **Requesting Extension to previously approved protocol.**
(Please provide: 1) abstract of findings or summary of progress to date, 2) reason for extension request, and 3) any changes being made to originally approved protocol.)

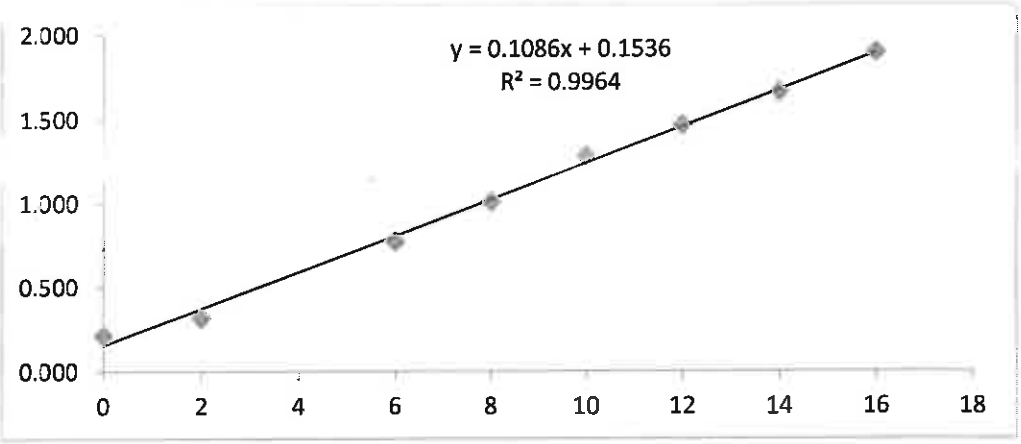
☐ **Requesting Addendum to previously approved protocol.**
(Please provide: 1) abstract of findings or summary of progress to date, 2) reason for addendum request, and 3) any changes being made to originally approved protocol.)

Submit electronic copy to jmu_grants@jmu.edu and a "signed" hard copy to the Office of Sponsored Programs, MSC 5728 by the due date noted on the Action of the Board Approval Form.

ELISA - LASIK P09 - P10 100 ng **4/2/13**

pLAC 3/20/13 = 237ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

JG	pLAC (ng)	abs 1	abs 2	abs 3	average
	16	1.849	1.908	1.927	1.895
	14	1.689	1.661	1.639	1.663
	12	1.416	1.485	1.498	1.466
	10	1.197	1.339	1.312	1.283
	8	0.999	1.023	1.006	1.009
	6	0.804	0.744	0.766	0.771
	4	0.156	0.123	0.123	
	2	0.316	0.326	0.311	0.318
	0	0.182	0.173	0.279	0.211



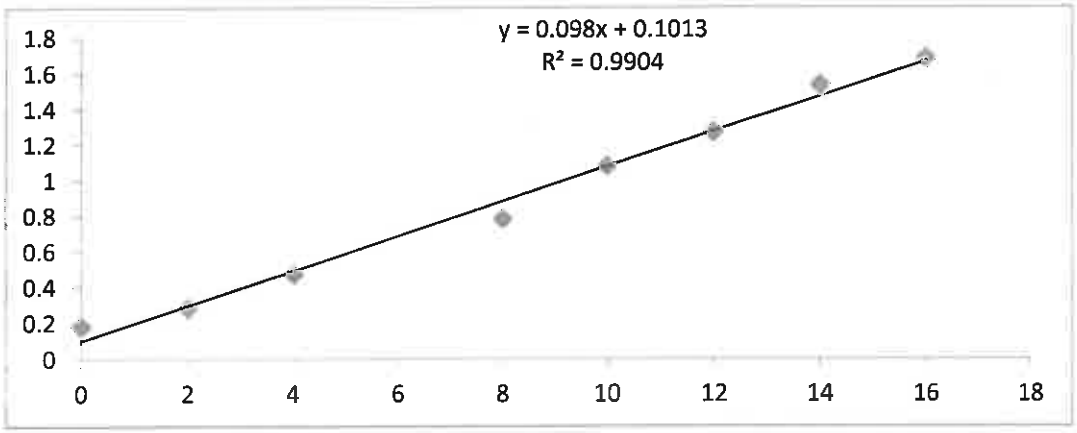
Tear	abs 1	abs 2	abs 3	average	% lacritin
P09 Preop	0.852	0.79	0.958	0.867	6.6
P09 1 DAY	0.941	1.023	0.993	0.986	7.7
P09 1 WK	1.313	1.371	1.301	1.328	10.8
P09 1 MO	0.73	0.822	0.827	0.793	5.9
P09 3 MO	1.248	1.357	1.386	1.330	10.8
P09 6 MO	1.426	1.472	1.332	1.410	11.6
P10 Preop	1.382	1.383	1.543	1.436	11.8
P10 1DAY	1.18	1.203	1.214	1.199	9.6
P10 1 WK	1.152	0.995	1.22	1.122	8.9
P10 1 MO	1.649	2.177	1.751	1.859	15.7
P10 3 MO	1.651	1.577	1.654	1.627	13.6
P10 6 MO	1.46	1.185	1.51	1.385	11.3

ELISA - LASIK P09 - P10 100 ng **4/2/13**

pLAC 3/20/13 = 371ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

KS

pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.781	1.424	1.844	1.683
14	1.484	1.538	1.579	1.533667
12	1.207	1.293	1.303	1.268
10	1.044	1.099	1.099	1.081
8	0.764	0.788	0.789	0.780
6	0.555	0.572	0.543	
4	0.49	0.474	0.445	0.470
2	0.271	0.28	0.292	0.281
0	0.185	0.175	0.181	0.180

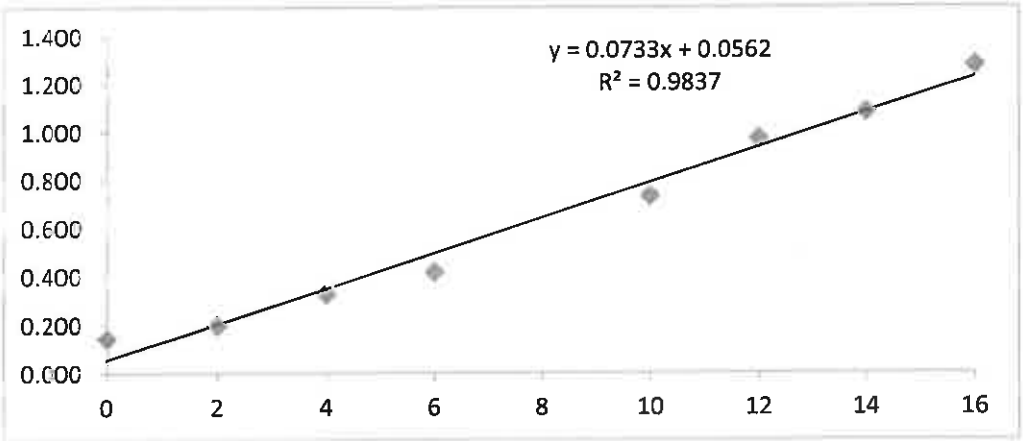


Tear	abs 1	abs 2	abs 3	average	% lacritin
P09 Preop	0.899	0.87	0.863	0.877	7.9
P09 1 DAY	0.924	0.922	0.902	0.916	8.3
P09 1 WK	1.29	1.287	1.262	1.280	12.0
P09 1 MO	0.791	0.777	0.841	0.803	7.2
P09 3 MO	1.253	1.24	1.238	1.244	11.7
P09 6 MO	1.294	1.338	1.276	1.303	12.3
P10 Preop	1.359	1.336	1.352	1.349	12.7
P10 1DAY	1.669	1.657	1.176	1.501	14.3
P10 1 WK	0.884	1.182	1.171	1.079	10.0
P10 1 MO	1.563	1.672	1.683	1.639	15.7
P10 3 MO	1.692	1.732	1.72	1.715	16.5
P10 6 MO	1.455	1.454	1.462	1.457	13.8

ELISA - LASIK P11-P12 100 ng **4/3/13**

pLAC 3/20/13 = 295ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

KS	pLAC (ng)	abs 1	abs 2	abs 3	average
	16	1.242	1.316	1.275	1.278
	14	1.058	1.088	1.094	1.08
	12	0.941	0.996	0.977	0.971
	10	0.714	0.738	0.743	0.732
	6	0.406	0.417	0.424	0.416
	4	0.326	0.333	0.321	0.327
	2	0.204	0.195	0.189	0.196
	0	0.151	0.142	0.135	0.143



Tear	abs 1	abs 2	abs 3	average	% lacritin
P11 PREOP	0.864	0.902	0.9	0.889	11.4
P11 1 DAY	0.488	0.488	0.492	0.489	5.9
P11 1 WK	0.887	0.87	0.902	0.886	11.3
P11 1 MO	0.957	0.926	0.929	0.937	12.0
P11 3 MO	0.569	0.627	0.628	0.608	7.5
P11 6 MO	0.578	0.556	0.567	0.567	7.0
P12 PREOP	0.854	0.861	0.871	0.862	11.0
P12 1 DAY	0.568	0.567	0.578	0.571	7.0
P12 1 WK	1.075	1.053	1.029	1.052	13.6
P12 1 MO	1.104	1.083	1.063	1.083	14.0
P12 3 MO	1.14	1.157	1.12	1.139	14.8
P12 6 MO	0.964	0.967	0.968	0.966	12.4

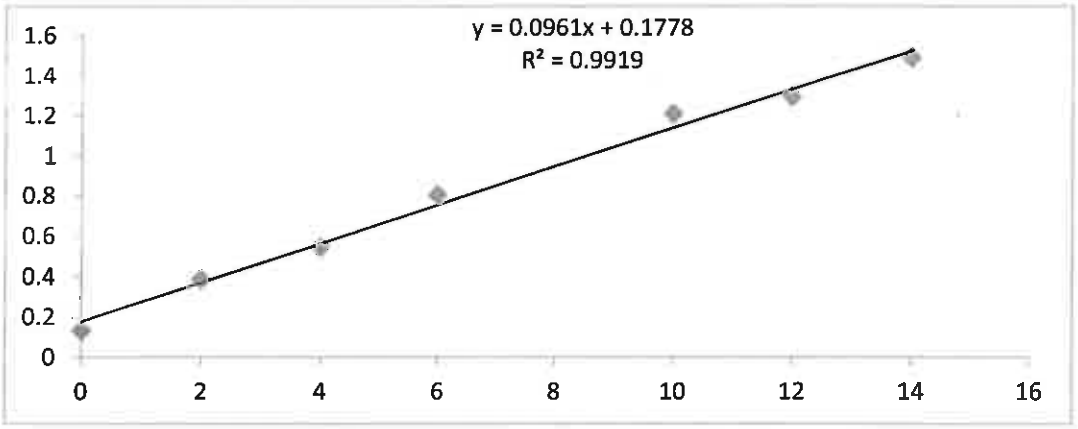
added 66uL of pLAC at 295 to 46 instead of 54 to 46
actually calculates to 16.2ng, 14.175ng, etc

ELISA - LASIK P11-P12 100 ng **4/3/13**

pLAC 3/20/13 = 295ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

JG

pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.432	1.442	1.442	1.438667
14	1.506	1.473	1.486	1.488333
12	1.29	1.299	1.287	1.292
10	1.19	1.195	1.238	1.208
6	0.823	0.787	0.803	0.804
4	0.521	0.559	0.559	0.546
2	0.406	0.383	0.373	0.387
0	0.145	0.125	0.125	0.132

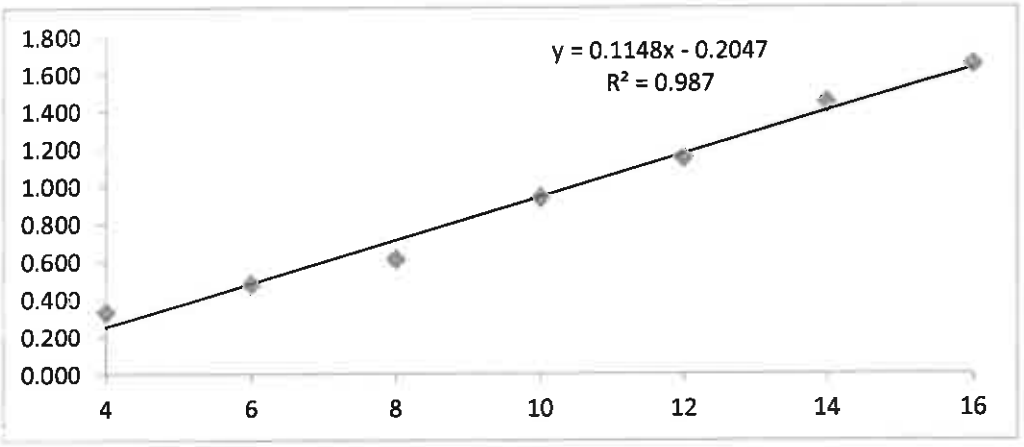


Tear	abs 1	abs 2	abs 3	average	% lacritin
P11 PREOP	1.014	1.038	1.021	1.024	8.8
P11 1 DAY	0.492	0.478	0.495	0.488	3.2
P11 1 WK	0.932	0.915	0.892	0.913	7.7
P11 1 MO	1.032	1.01	1.035	1.026	8.8
P11 3 MO	0.659	0.647	0.666	0.657	5.0
P11 6 MO	0.608	0.596	0.629	0.611	4.5
P12 PREOP	0.923	0.903	0.904	0.910	7.6
P12 1 DAY	0.628	0.567	0.585	0.593	4.3
P12 1 WK	1.082	1.069	1.062	1.071	9.3
P12 1 MO	1.108	1.102	1.047	1.086	9.4
P12 3 MO	1.191	1.158	1.127	1.159	10.2
P12 6 MO	0.953	0.962	0.929	0.948	8.0

ELISA - LASIK P13 - P14 100 ng **4/2/13**

pLAC 3/20/13 = 304 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

AT	pLAC (ng)	abs 1	abs 2	abs 3	average
	16	1.661	1.634	1.658	1.651
	14	1.445	1.395	1.502	1.447
	12	1.087	1.124	1.234	1.148
	10	0.86	0.886	1.067	0.938
	8	0.562	0.589	0.682	0.611
	6	0.468	0.475	0.495	0.479
	4	0.347	0.323	0.325	0.332
	2	0.306	0.319	0.274	0.300
	0	0.332	0.311	0.274	0.306



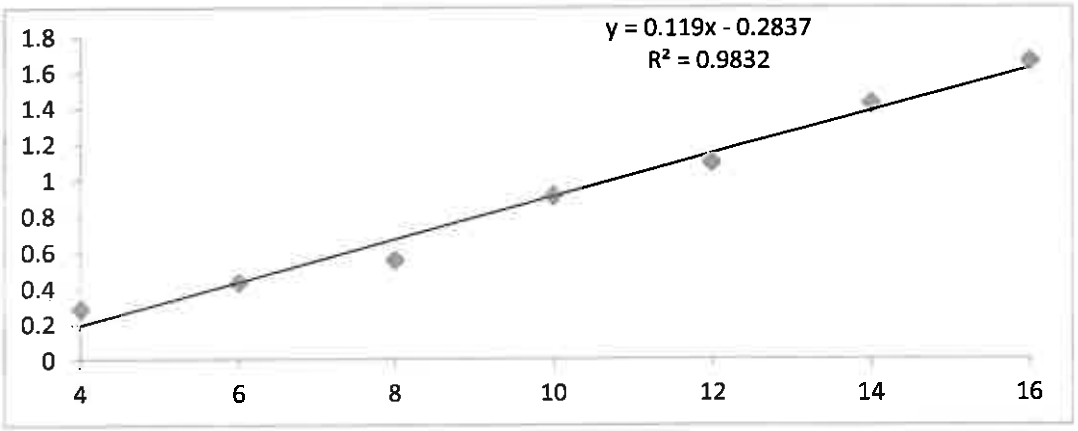
Tear	abs 1	abs 2	abs 3	average	% lacritin
P13 PREOP	1.196	1.24	1.23	1.222	12.4
P13 1 DAY	0.688	0.652	0.685	0.675	7.7
P13 1 WK	0.985	0.981	0.978	0.981	10.3
P13 1 MO	1.196	1.177	1.133	1.169	12.0
P13 3 MO	0.744	0.685	0.654	0.694	7.8
P13 6 MO	1.18	1.136	1.107	1.141	11.7
P14 PREOP	1.45	1.431	1.398	1.426	14.2
P14 1 DAY	1.286	1.282	1.163	1.244	12.6
P14 1 WK	1.146	1.207	1.222	1.192	12.2
P14 1 MO	1.019	1.025	1.033	1.026	10.7
P14 3 MO	1.305	1.368	1.425	1.366	13.7

ELISA - LASIK P13 - P14 100 ng **4/2/13**

pLAC 3/20/13 = 304 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

KS

pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.685	1.637	1.661	1.661
14	1.407	1.424	1.438	1.423
12	1.058	1.114	1.103	1.092
10	0.912	0.933	0.879	0.908
8	0.557	0.552	0.55	0.553
6	0.462	0.42	0.407	0.430
4	0.29	0.264	0.288	0.281
2	0.218	0.217	0.19	0.208
0	0.152	0.155	0.149	0.152

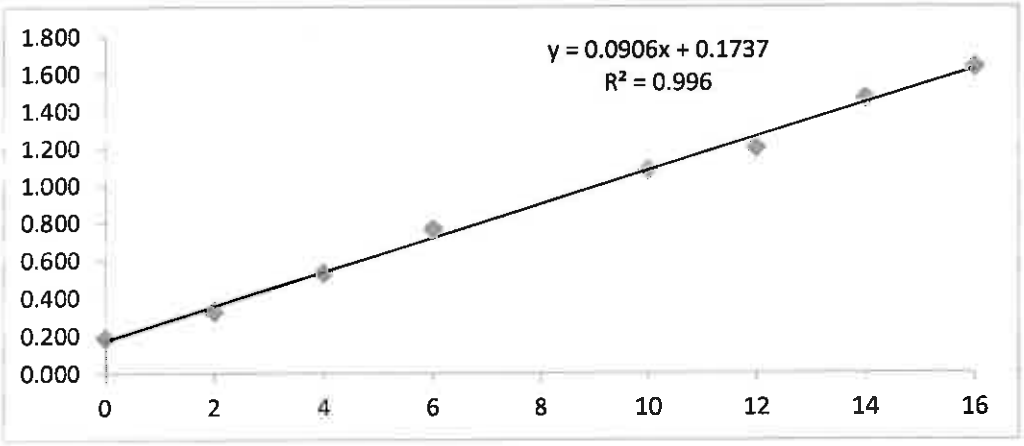


Tear	abs 1	abs 2	abs 3	average	% lacritin
P13 PREOP	1.111	1.179	1.141	1.144	12.0
P13 1 DAY	0.613	0.622	0.643	0.626	7.6
P13 1 WK	1.025	1.071	0.973	1.023	11.0
P13 1 MO	1.202	1.187	1.199	1.196	12.4
P13 3 MO	0.731	0.737	0.695	0.721	8.4
P13 6 MO	1.093	1.08	1.072	1.082	11.5
P14 PREOP	1.341	1.364	1.369	1.358	13.8
P14 1 DAY	1.384	1.259	1.26	1.301	13.3
P14 1 WK	1.276	1.283	1.281	1.280	13.1
P14 1 MO	1.092	1.083	1.066	1.080	11.5
P14 3 MO	1.291	1.38	1.408	1.360	13.8

ELISA - LASIK P15 - P16 100 ng **4/9/13**

pLAC 3/20/13 = 253 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

AT	pLAC (ng)	abs 1	abs 2	abs 3	average
	16	1.634	1.649	1.621	1.635
	14	1.442	1.502	1.458	1.467
	12	1.188	1.17	1.241	1.200
	10	1.077	1.054	1.117	1.083
	6	0.764	0.795	0.732	0.764
	4	0.543	0.523	0.522	0.529
	2	0.321	0.337	0.316	0.325
	0	0.19	0.192	0.181	0.188



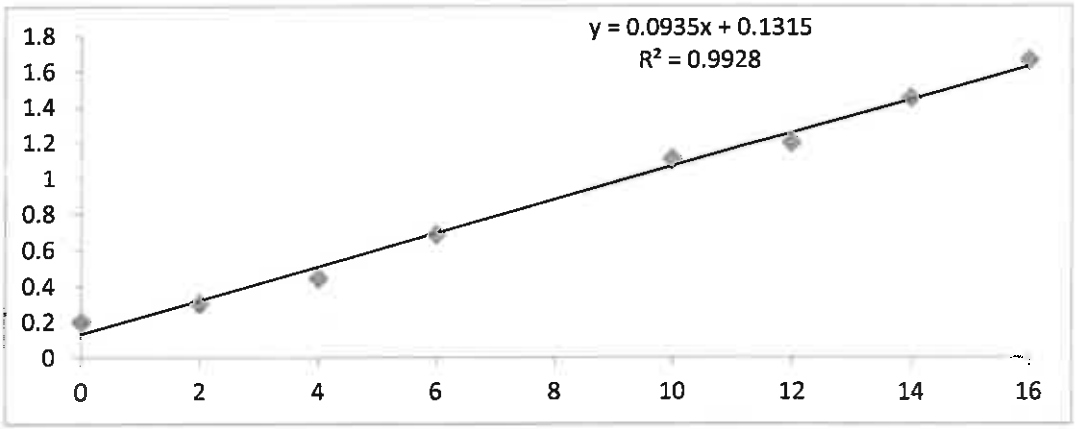
Tear	abs 1	abs 2	abs 3	average	% lacritin
P15 PREOP	0.811	0.846	0.793	0.817	7.1
P15 1 DAY	1.369	1.416	1.423	1.403	13.6
P15 1 WK	1.558	1.533	1.605	1.565	15.4
P15 1 MO	0.973	0.947	1.037	0.986	9.0
P15 3 MO	1.545	1.53	1.506	1.527	14.9
P15 6 MO	1.059	1.061	1.036	1.052	9.7
P16 PREOP	1.462	1.425	1.412	1.433	13.9
P16 1 DAY	0.225	0.233	0.248	0.235	0.7
P16 1 WK	1.291	1.278	0.982	1.184	11.1
P16 1 MO	0.227	0.241	0.238	0.235	0.7
P16 3 MO	1.285	1.333	1.254	1.291	12.3
P16 6 MO	1.193	1.197	1.153	1.181	11.1

ELISA - LASIK P15 - P16 100 ng **4/9/13**

pLAC 3/20/13 = 304 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

KS

pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.673	1.679	1.631	1.661
14	1.467	1.462	1.417	1.448667
12	1.204	1.176	1.21	1.197
10	1.079	1.115	1.121	1.105
6	0.687	0.668	0.699	0.685
4	0.459	0.432	0.43	0.440
2	0.297	0.303	0.301	0.300
0	0.206	0.197	0.197	0.200

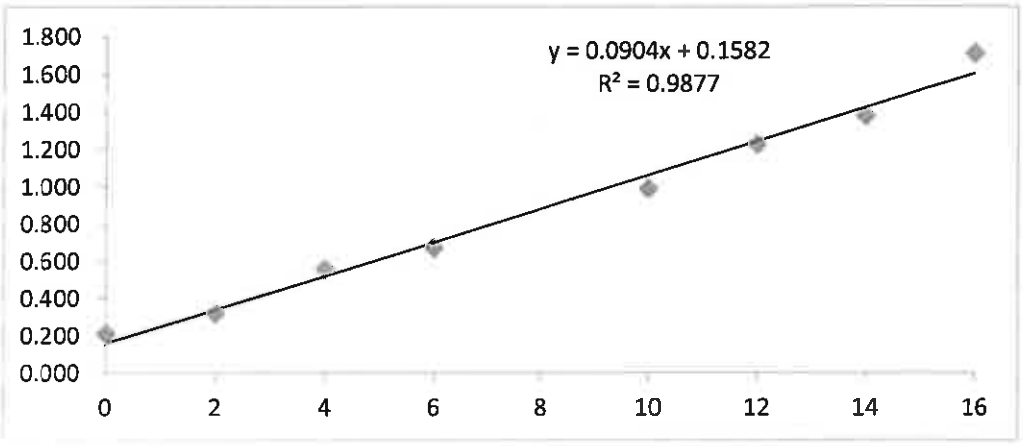


Tear	abs 1	abs 2	abs 3	average	% lacritin
P15 PREOP	0.794	0.68	0.782	0.752	6.6
P15 1 DAY	1.367	1.405	1.371	1.381	13.4
P15 1 WK	1.539	1.574	1.545	1.553	15.2
P15 1 MO	1.008	1	1.024	1.011	9.4
P15 3 MO	1.563	1.544	1.593	1.567	15.3
P15 6 MO	1.023	1.057	1.072	1.051	9.8
P16 PREOP	1.409	1.302	1.47	1.394	13.5
P16 1 DAY	0.243	0.263	0.279	0.262	1.4
P16 1 WK	1.27	1.25	1.266	1.262	12.1
P16 1 MO	0.218	0.219	0.239	0.225	1.0
P16 3 MO	1.302	1.38	1.347	1.343	13.0
P16 6 MO	1.17	1.162	1.162	1.165	11.0

ELISA - LASIK P17 - P18 100 ng **4/9/13**

pLAC 3/20/13 = 304 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

AT	pLAC (ng)	abs 1	abs 2	abs 3	average
	16	1.66	1.733	1.736	1.710
	14	1.476	1.36	1.298	1.378
	12	1.224	1.195	1.26	1.226
	10	0.989	0.961	1.012	0.987
	6	0.716	0.699	0.589	0.668
	4	0.536	0.644	0.493	0.558
	2	0.309	0.329	0.313	0.317
	0	0.207	0.205	0.21	0.207



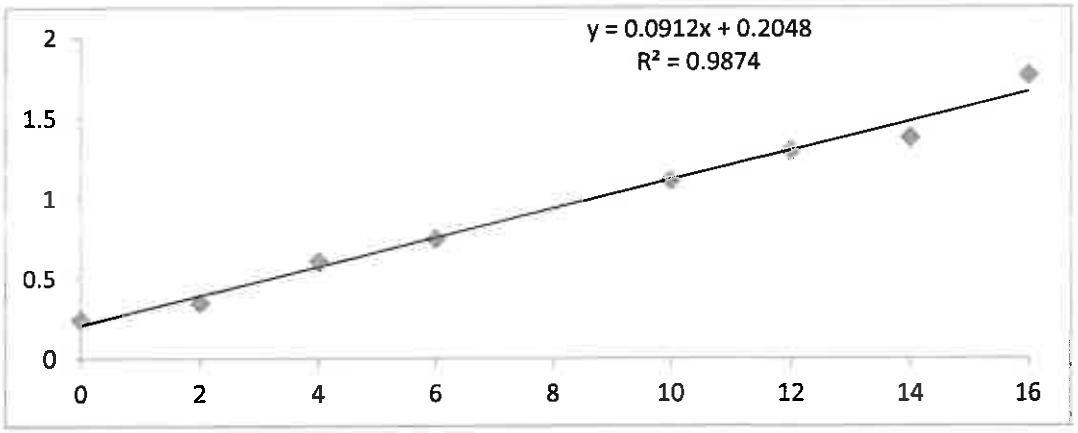
Tear	abs 1	abs 2	abs 3	average	% lacritin
P17 PREOP	1.233	1.084	1.127	1.148	10.9
P17 1 DAY	0.544	0.536	0.537	0.539	4.2
P17 1 WK	1.798	1.092	1.088	1.326	12.9
P17 1 MO	1.14	1.138	1.044	1.107	10.5
P17 3 MO	0.869	0.956	0.878	0.901	8.2
P17 6 MO	0.678	0.804	1.116	0.866	7.8
P18 PREOP	1.356	1.216	1.879	1.484	14.7
P18 1 DAY	1.229	1.215	1.249	1.231	11.9
P18 1 WK	1.166	1.712	1.033	1.304	12.7
P18 1 MO	0.873	1.497	0.704	1.025	9.6
P18 3 MO	1.219	1.166	1.135	1.173	11.2

ELISA - LASIK P17 - P18 100 ng **4/9/13**

pLAC 3/20/13 = 304 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

JG

pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.763	1.789	1.738	1.763333
14	1.518	1.498	1.111	1.375667
12	1.301	1.317	1.269	1.296
10	1.095	1.103	1.131	1.110
6	0.629	0.763	0.84	0.744
4	0.601	0.592	0.615	0.603
2	0.357	0.326	0.349	0.344
0	0.219	0.212	0.287	0.239



Tear	abs 1	abs 2	abs 3	average	% lacritin
P17 PREOP	1.283	1.284	1.131	1.233	11.3
P17 1 DAY	0.54	0.541	1.121	0.734	5.8
P17 1 WK	1.105	1.124	1.225	1.151	10.4
P17 1 MO	1.161	1.148	1.155	1.155	10.4
P17 3 MO	0.897	0.939	1.011	0.949	8.2
P17 6 MO	0.672	0.555	0.681	0.636	4.7
P18 PREOP	1.486	1.43	1.368	1.428	13.4
P18 1 DAY	1.263	1.162	1.373	1.266	11.6
P18 1 WK	1.205	1.123	1.106	1.145	10.3
P18 1 MO	0.883	0.832	0.884	0.866	7.3
P18 3 MO	1.332	1.241	1.431	1.335	12.4

ELISA - LASIK P20- P21 100 ng **7/3/13**

pLAC 5/29/13 = 321 ug/mL

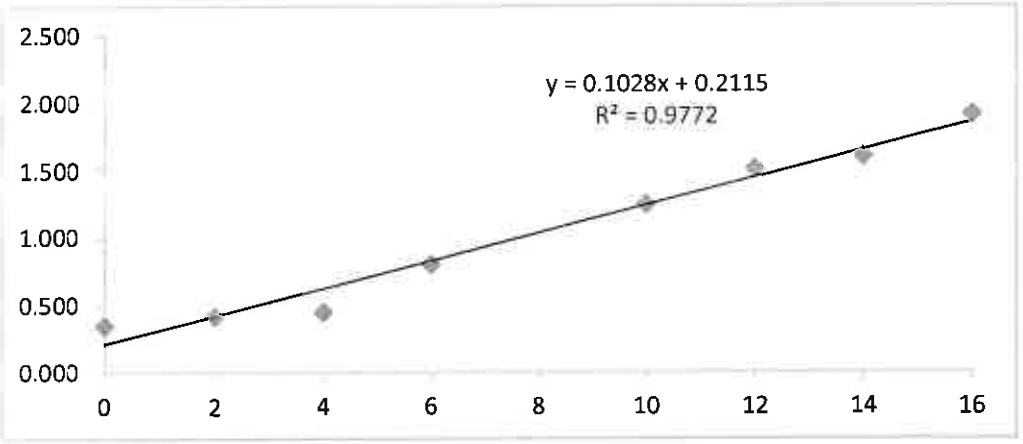
Blocking buffer = 1% BSA

Primary antibody = 1:400 Final Bleed 5529

Secondary antibody = 1:800 goat anti rabbit HRP

Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.845	1.934	1.946	1.908
14	1.596	1.59	1.603	1.596
12	1.47	1.494	1.548	1.504
10	1.213	1.256	1.261	1.243
6	0.785	0.827	0.796	0.803
4	0.453	0.434	0.455	0.447
2	0.426	0.404	0.425	0.418
0	0.34	0.358	0.345	0.348

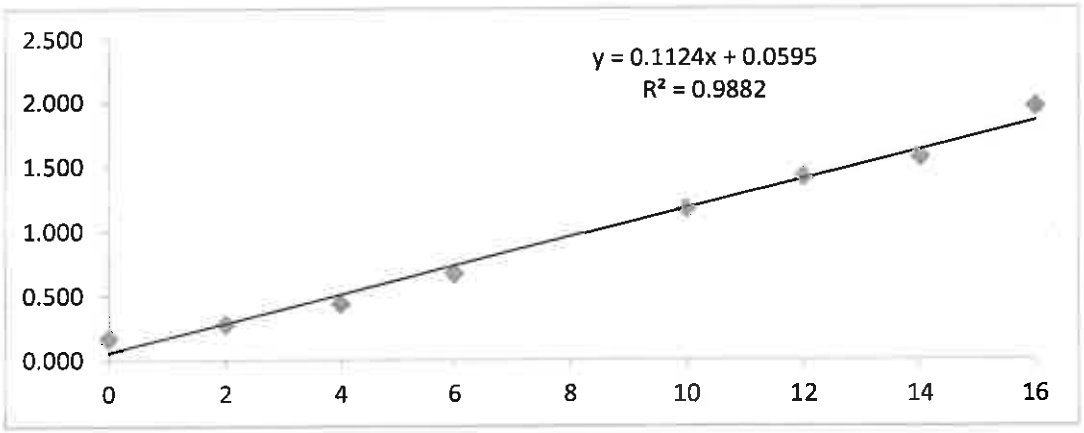


Tear	abs 1	abs 2	abs 3	average	% lacritin
P20 PRE	1.371	1.053	1.349	1.258	10.2
P20 1 DAY	1.903	1.895	1.985	1.928	16.7
P20 1 WEEK	1.374	1.435	1.401	1.403	11.6
P20 1 MO	1.058	1.161	1.095	1.105	8.7
P20 3 MO	1.351	1.358	1.348	1.352	11.1
P20 6 MO	1.613	1.666	1.643	1.641	13.9
P21 PRE	1.475	1.418	1.493	1.462	12.2
P21 1 DAY	1.113	1.105	1.108	1.109	8.7
P21 1 WEEK	1.496	1.754	1.657	1.636	13.9
P21 1 MO	1.11	1.036	1.165	1.104	8.7
P21 3 MO	1.378	1.4	1.446	1.408	11.6
P21 6 MO	1.332	1.449	1.39	1.390	11.5

ELISA - LASIK P20- P21 100 ng **7/3/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.956	1.966	1.96	1.961
14	1.575	1.566	1.56	1.567
12	1.393	1.444	1.412	1.416
10	1.135	1.172	1.201	1.169
6	0.649	0.703	0.662	0.671
4	0.434	0.434	0.446	0.438
2	0.283	0.265	0.284	0.277
0	0.18	0.165	0.164	0.170

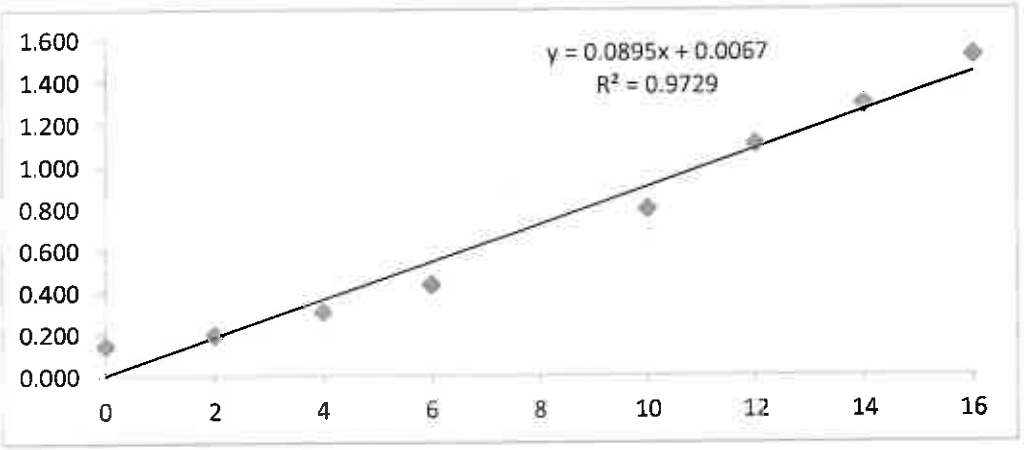


Tear	abs 1	abs 2	abs 3	average	% lacritin
P20 PRE	1.331	1.305	1.24	1.292	11.4
P20 1 DAY	1.883	1.843	1.774	1.833	16.3
P20 1 WEEK	1.496	1.469	1.397	1.454	12.9
P20 1 MO	1.158	1.04	1.078	1.092	9.7
P20 3 MO	1.259	1.315	1.244	1.273	11.3
P20 6 MO	1.616	1.544	1.519	1.560	13.8
P21 PRE	1.371	1.321	1.33	1.341	11.9
P21 1 DAY	1.004	0.986	0.963	0.984	8.7
P21 1 WEEK	1.657	1.601	1.6	1.619	14.4
P21 1 MO	1.146	1.231	1.089	1.155	10.2
P21 3 MO	1.422	1.364	1.378	1.388	12.3
P21 6 MO	1.34	1.437	1.43	1.402	12.4

ELISA - LASIK P22- P24 100 ng **7/9/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.518	1.508	1.526	1.517
14	1.277	1.297	1.297	1.290
12	1.092	1.093	1.118	1.101
10	0.792	0.787	0.808	0.796
6	0.427	0.427	0.45	0.435
4	0.305	0.289	0.316	0.303
2	0.201	0.195	0.185	0.194
0	0.151	0.138	0.144	0.144

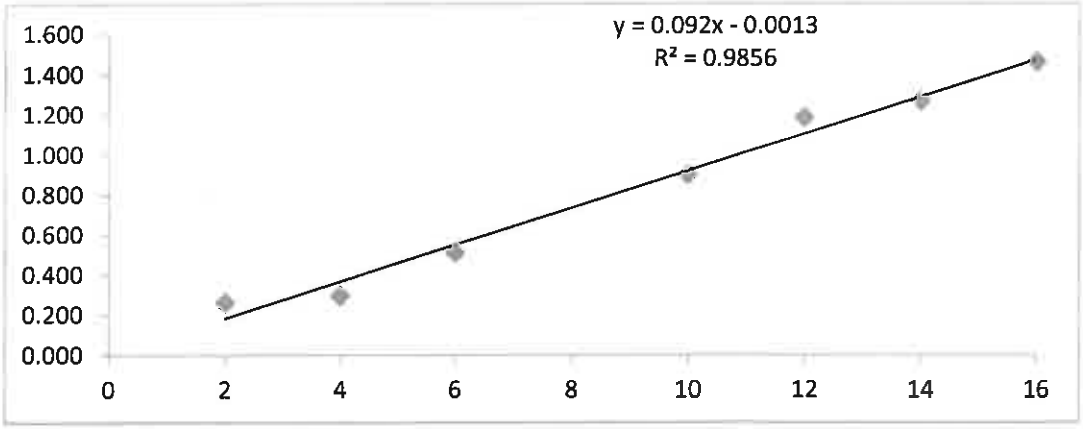


Tear	abs 1	abs 2	abs 3	average	% lacritin
P22 PRE	1.172	1.027	1.076	1.092	12.1
P22 1 DAY	0.87	0.792	0.84	0.834	9.2
P22 1 WEEK	0.877	0.881	0.798	0.852	9.4
P22 1 MO	0.857	0.851	0.872	0.860	9.5
P22 3 MO	0.966	0.939	0.961	0.955	10.6
P22 6 MO	0.555	0.4	0.506	0.487	5.4
P24 PRE	0.962	0.723	0.912	0.866	9.6
P24 1 DAY	0.773	0.791	0.714	0.759	8.4
P24 1 WEEK	1.212	1.197	1.13	1.180	13.1
P24 1 MO	0.882	0.867	0.309	0.875	9.7
P24 3 MO	1.062	1.081	1.045	1.063	11.8
P24 6 MO	1.182	1.137	1.2	1.173	13.0

ELISA - LASIK P22- P24 100 ng **7/9/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.44	1.504	1.438	1.461
14	1.235	1.252	1.315	1.267
12	1.131	1.192	1.232	1.185
10	0.82	0.936	0.939	0.898
6	0.412	0.552	0.57	0.511
4	0.305	0.293	0.284	0.294
2	0.303	0.172	0.307	0.261
0	0.296	0.294	0.303	0.298

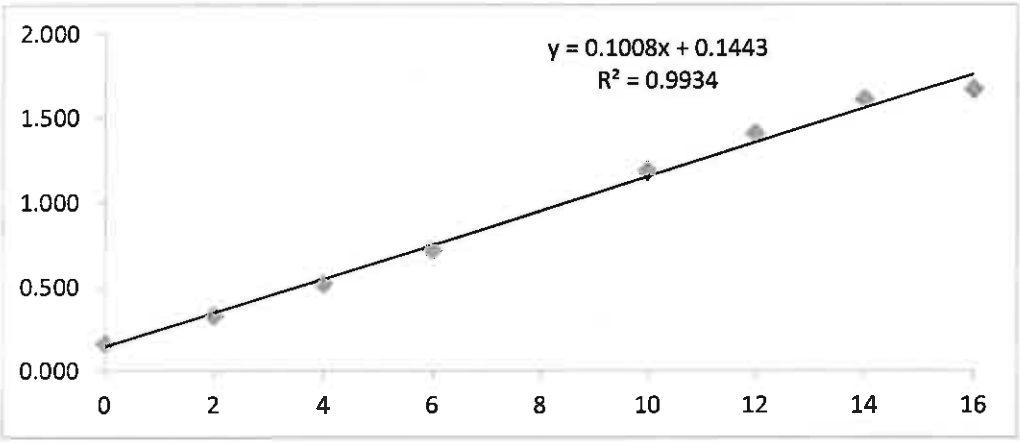


Tear	abs 1	abs 2	abs 3	average	% lacritin
P22 PRE	1.291	1.279	1.295	1.288	14.0
P22 1 DAY	0.994	0.951	0.951	0.965	10.5
P22 1 WEEK	0.986	1.017	1.012	1.005	10.9
P22 1 MO	0.839	0.804	0.861	0.835	9.1
P22 3 MO	0.918	1.035	1.02	0.991	10.8
P22 6 MO	0.593	0.681	0.706	0.660	7.2
P24 PRE	0.964	1.073	1.057	1.031	11.2
P24 1 DAY	0.764	0.851	0.972	0.862	9.4
P24 1 WEEK	1.285	1.214	1.151	1.217	13.2
P24 1 MO	0.763	0.864	0.862	0.830	9.0
P24 3 MO	1.053	1.088	1.098	1.080	11.7
P24 6 MO	1.189	1.212	1.237	1.213	13.2

ELISA - LASIK P23- P34 100 ng **7/18/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.695	1.648	1.666	1.670
14	1.602	1.605	1.616	1.608
12	1.402	1.405	1.414	1.407
10	1.158	1.183	1.219	1.187
6	0.705	0.719	0.747	0.724
4	0.435	0.583	0.545	0.521
2	0.339	0.329	0.325	0.331
0	0.162	0.159	0.162	0.161

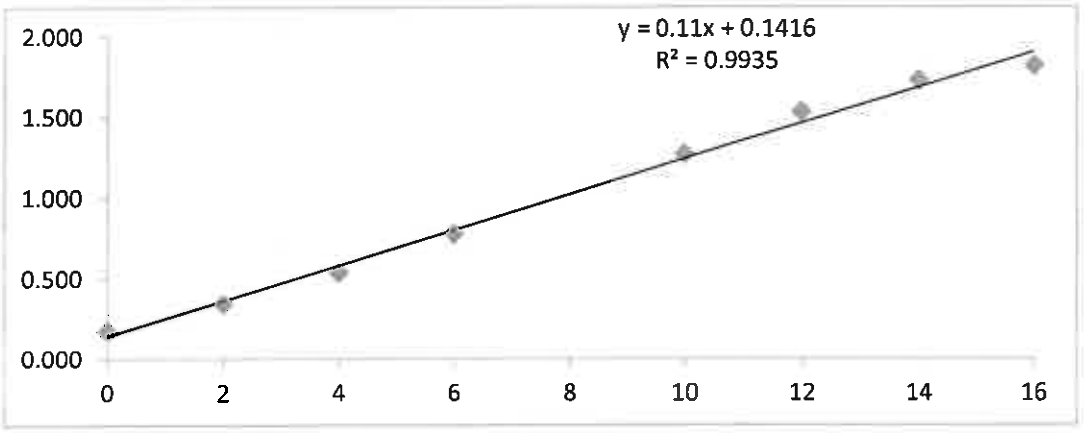


Tear	abs 1	abs 2	abs 3	average	% lacritin
P23 PRE	1.111	1.123	1.095	1.110	9.6
P23 1 DAY	0.426	0.44	0.424	0.430	2.8
P23 1 WK	1.516	1.589	1.51	1.538	13.8
P23 1 MO	1.577	1.605	1.593	1.592	14.4
P23 3 MO	0.747	0.724	0.732	0.734	5.9
P23 6 MO	1.498	1.494	1.487	1.493	13.4
P34 PRE	1.511	1.458	1.453	1.474	13.2
P34 1 DAY	1.346	1.314	1.242	1.301	11.5
P34 1 WEEK	1.419	1.399	1.422	1.413	12.6
P34 1 MO	1.299	1.288	1.187	1.258	11.0
P34 3 MO	1.34	1.36	1.416	1.372	12.2
P34 6 MO	1.699	1.746	1.539	1.661	15.0

ELISA - LASIK P23- P34 100 ng **7/18/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.816	1.803	1.823	1.814
14	1.707	1.755	1.72	1.727
12	1.515	1.557	1.52	1.531
10	1.261	1.264	1.284	1.270
6	0.767	0.788	0.779	0.778
4	0.543	0.539	0.532	0.538
2	0.346	0.343	0.35	0.345
0	0.171	0.169	0.171	0.170



Tear	abs 1	abs 2	abs 3	average	% lacritin
P23 PRE	1.25	1.284	1.238	1.257	10.1
P23 1 DAY	0.483	0.482	0.476	0.480	3.1
P23 1 WK	1.666	1.676	1.637	1.660	13.8
P23 1 MO	1.762	1.573	1.678	1.671	13.9
P23 3 MO	0.817	0.83	0.787	0.811	6.1
P23 6 MO	1.656	1.692	1.665	1.671	13.9
P34 PRE	1.625	1.597	1.58	1.601	13.3
P34 1 DAY	1.466	1.481	1.451	1.466	12.0
P34 1 WEEK	1.527	1.523	1.528	1.526	12.6
P34 1 MO	1.413	1.367	1.297	1.359	11.1
P34 3 MO	1.505	1.528	1.449	1.840	15.4
P34 6 MO	1.84	1.829	1.734	1.801	15.1

ELISA - LASIK P25- P26 100 ng **7/10/13**

pLAC 5/29/13 = 321 ug/mL

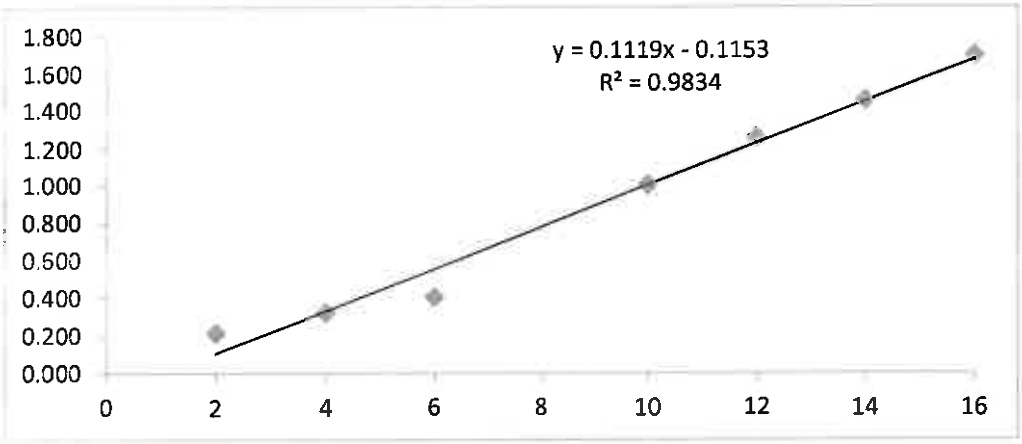
Blocking buffer = 1% BSA

Primary antibody = 1:400 Final Bleed 5529

Secondary antibody = 1:800 goat anti rabbit HRP

Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.696	1.709	1.685	1.697
14	1.436	1.474	1.459	1.456
12	1.237	1.261	1.262	1.253
10	0.939	1.046	1.025	1.003
6	0.386	0.421	0.411	0.406
4	0.309	0.318	0.342	0.323
2	0.213	0.222	0.212	0.216
0	0.169	0.156	0.166	0.164

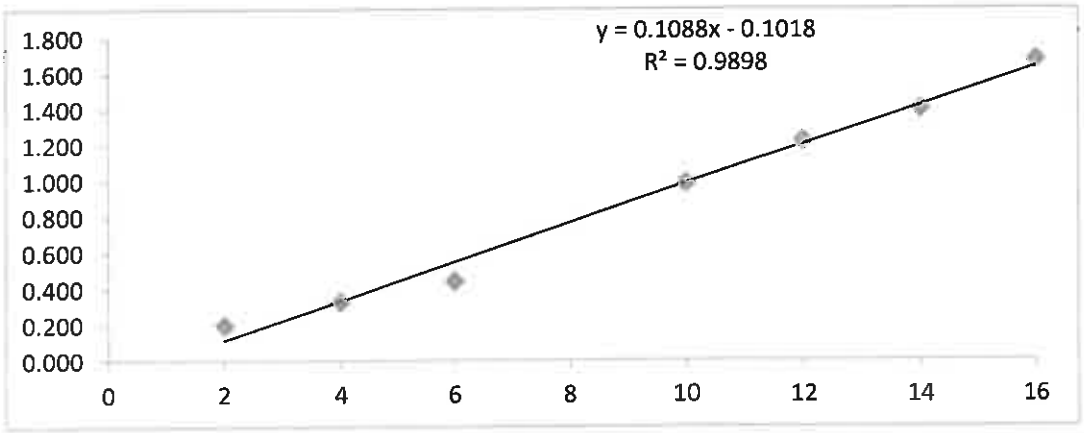


Tear	abs 1	abs 2	abs 3	average	% lacritin
P25 PRE	0.603	0.618	0.571	0.597	6.4
P25 1 DAY	0.95	0.985	0.938	0.958	9.6
P25 1 WK	1.051	0.997	0.997	1.015	10.1
P25 1 MO	0.739	0.705	0.734	0.726	7.5
P25 3 MO	0.719	0.71	0.699	0.709	7.4
P25 6 MO	0.953	0.972	0.945	0.957	9.6
P26 PRE	1.125	1.141	1.115	1.127	11.1
P26 1 DAY	0.971	1.009	0.984	0.988	9.9
P26 1 WEEK	1.051	1.057	1.012	1.040	10.3
P26 1 MO	1.276	1.267	1.314	1.272	12.4
P26 3 MO	1.114	1.097	1.117	1.109	10.9
P26 6 MO	1.207	1.27	1.204	1.227	12.0

ELISA - LASIK P25- P26 100 ng **7/10/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.63	1.718	1.677	1.675
14	1.407	1.403	1.396	1.402
12	1.226	1.229	1.213	1.223
10	0.946	0.99	1.017	0.984
6	0.414	0.428	0.485	0.442
4	0.318	0.328	0.337	0.328
2	0.215	0.178	0.59	0.197
0	0.178	0.315	0.335	0.276

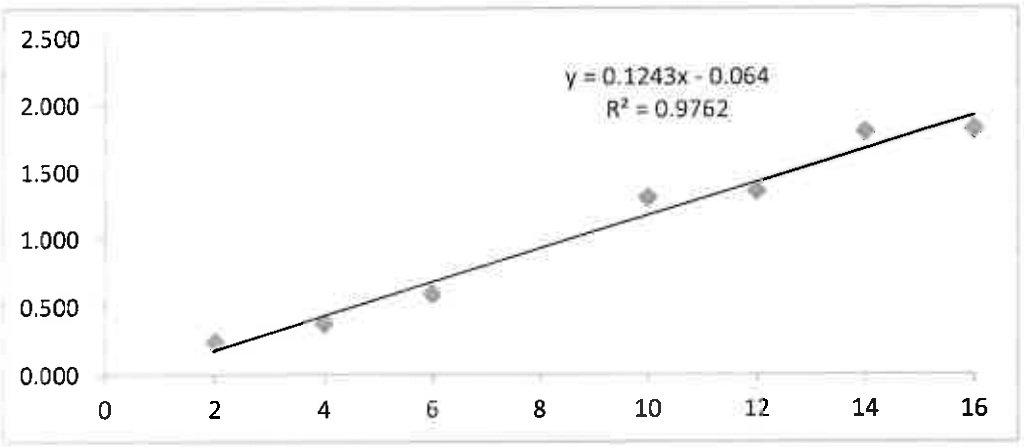


Tear	abs 1	abs 2	abs 3	average	% lacritin
P25 PRE	0.59	0.776	0.757	0.708	7.4
P25 1 DAY	0.942	0.927	1.14	1.003	10.2
P25 1 WK	1.022	1.05	1.033	1.035	10.4
P25 1 MO	0.698	0.724	0.747	0.723	7.6
P25 3 MO	0.735	0.763	0.713	0.737	7.7
P25 6 MO	0.955	1.112	0.95	1.006	10.2
P26 PRE	1.215	1.174	1.015	1.135	11.4
P26 1 DAY	1.062	1.01	0.864	0.979	9.9
P26 1 WEEK	1.119	0.999	1.02	1.046	10.5
P26 1 MO	1.218	1.242	1.238	1.233	12.3
P26 3 MO	1.009	0.993	0.995	0.999	10.1
P26 6 MO	1.14	1.127	1.118	1.128	11.3

ELISA - LASIK P27- P28 100 ng **7/11/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.817	1.838	1.82	1.825
14	1.801	1.809	1.79	1.800
12	1.363	1.357	1.354	1.358
10	1.284	1.347	1.305	1.312
6	0.597	0.583	0.597	0.592
4	0.37	0.378	0.376	0.375
2	0.239	0.256	0.235	0.243
0	0.187	0.187	0.188	0.187

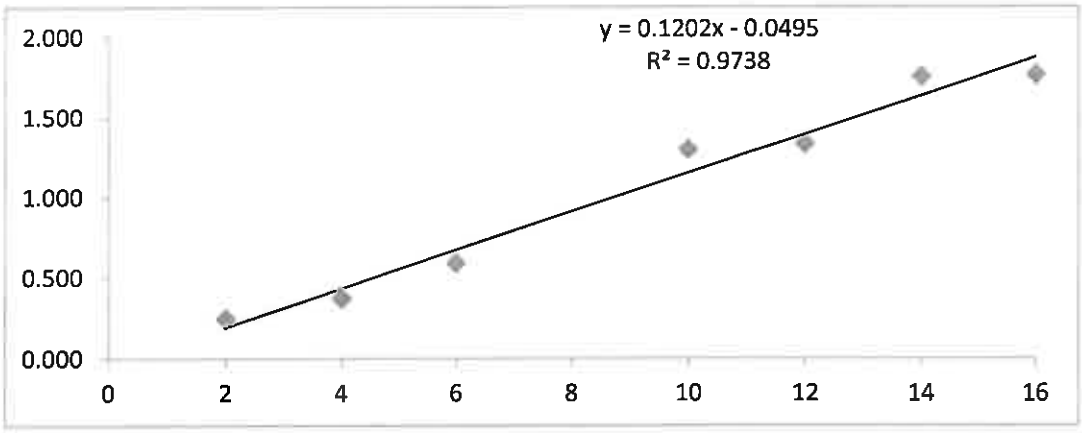


Tear	abs 1	abs 2	abs 3	average	% lacritin
P27 PRE	1.457	1.43	1.374	1.420	11.9
P27 1 DAY	1.704	1.733	1.729	1.722	14.4
P27 1 WK	1.834	1.794	1.899	1.842	15.3
P27 1 MO	1.474	1.502	1.509	1.495	12.5
P27 3 MO	1.477	1.469	1.455	1.467	12.3
P27 6 MO	1.61	1.578	1.628	1.605	13.4
P28 PRE	1.615	1.584	1.583	1.594	13.3
P28 1 DAY	1.812	1.881	1.94	1.878	15.6
P28 1 WEEK	1.591	1.559	1.561	1.570	13.1
P28 1 MO	1.431	1.419	1.448	1.433	12.0
P28 3 MO	1.595	1.661	1.54	1.599	13.4
P28 6 MO	1.748	1.73	1.709	1.729	14.4

ELISA - LASIK P27- P28 100 ng **7/11/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.776	1.766	1.747	1.763
14	1.790	1.73	1.738	1.753
12	1.322	1.35	1.326	1.333
10	1.24	1.314	1.337	1.297
6	0.556	0.604	0.598	0.586
4	0.355	0.363	0.392	0.370
2	0.239	0.245	0.395	0.242
0	0.184	0.319	0.32	0.274

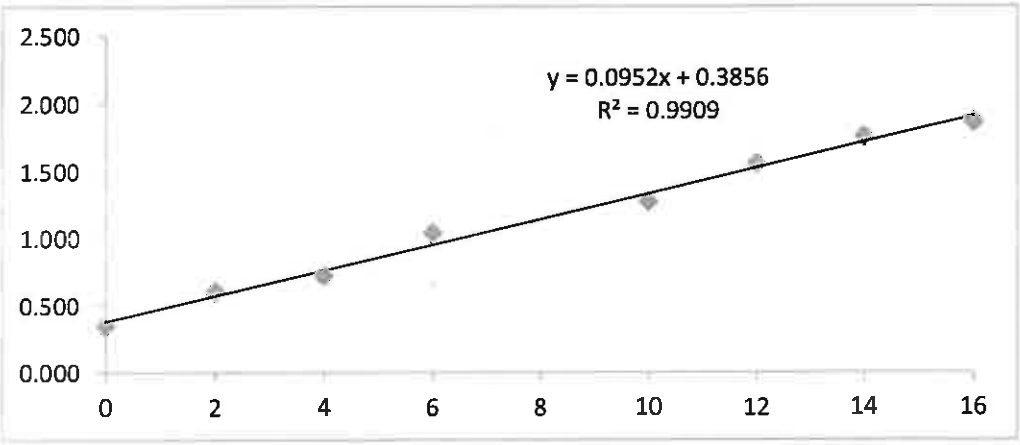


Tear	abs 1	abs 2	abs 3	average	% lacritin
P27 PRE	1.333	1.353	1.357	1.348	11.6
P27 1 DAY	1.713	1.671	1.546	1.643	14.1
P27 1 WK	1.825	1.736	1.769	1.777	15.2
P27 1 MO	1.462	1.446	1.468	1.459	12.5
P27 3 MO	1.518	1.527	1.408	1.484	12.8
P27 6 MO	1.542	1.537	1.539	1.539	13.2
P28 PRE	1.522	1.657	1.739	1.639	14.1
P28 1 DAY	1.828	1.882	1.936	1.882	16.1
P28 1 WEEK	1.546	1.614	1.548	1.569	13.5
P28 1 MO	1.478	1.453	1.467	1.466	12.6
P28 3 MO	1.674	1.561	1.601	1.612	13.8
P28 6 MO	1.664	1.694	1.637	1.665	14.3

ELISA - LASIK P29 - P30 100 ng **8/20/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.756	1.902	1.934	1.864
14	1.711	1.803	1.762	1.759
12	1.578	1.611	1.48	1.556
10	1.234	1.302	1.294	1.277
6	1.009	1.059	1.063	1.044
4	0.708	0.671	0.785	0.721
2	0.627	0.596	0.597	0.607
0	0.356	0.337	0.353	0.349

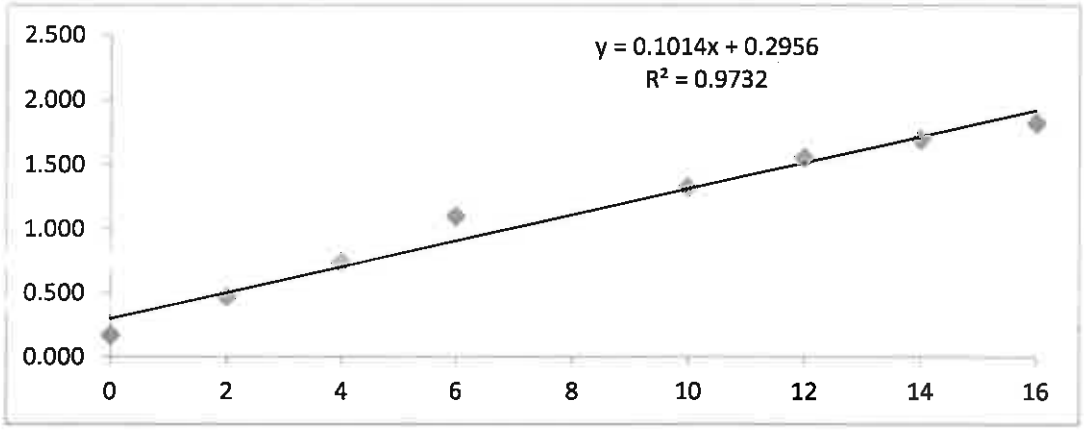


Tear	abs 1	abs 2	abs 3	average	% lacritin
P29 PRE	1.439	1.433	1.592	1.488	11.6
P29 1 DAY	1.454	1.505	1.565	1.508	11.8
P29 1 WEEK	1.387	1.526	1.582	1.498	11.7
P29 1 MO	1.338	1.362	1.365	1.355	10.2
P29 3 MO	1.608	1.502	1.486	1.532	12.0
P29 6 MO	1.363	1.164	1.196	1.241	9.0
P30 PRE	1.56	1.562	1.414	1.512	11.8
P30 1 DAY	1.494	1.488	1.526	1.503	11.7
P30 1WEEK	1.497	1.56	1.615	1.557	12.3
P30 1 MO	1.37	1.405	1.391	1.389	10.5
P30 3 MO	0.822	1.214	0.847	0.961	6.0
P30 6 MO	1.2	1.189	1.184	1.191	8.5

ELISA - LASIK P29 - P30 100 ng **8/20/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.829	1.828	1.805	1.821
14	1.688	1.717	1.68	1.695
12	1.554	1.55	1.551	1.552
10	1.322	1.318	1.33	1.323
6	1.085	1.116	1.086	1.096
4	0.737	0.716	0.765	0.739
2	0.464	0.464	0.462	0.464
0	0.163	0.168	0.164	0.165

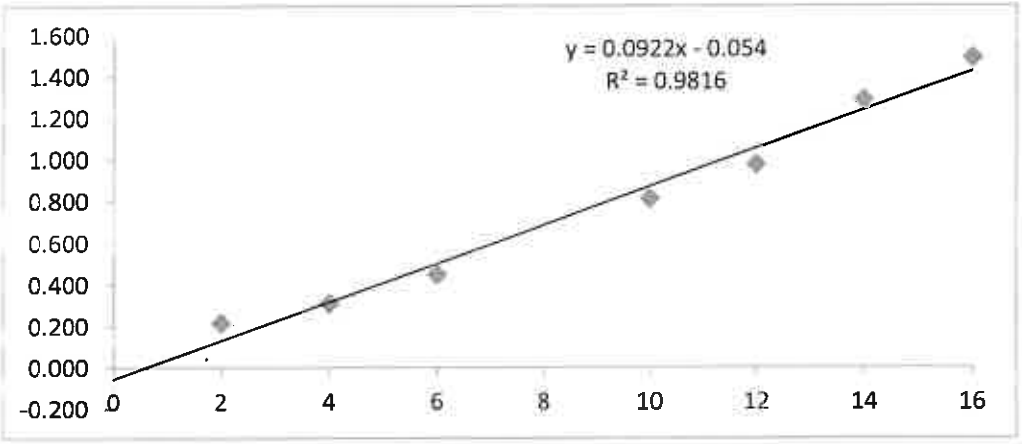


Tear	abs 1	abs 2	abs 3	average	% lacritin
P29 PRE	1.505	1.483	1.451	1.480	11.7
P29 1 DAY	1.535	1.504	1.504	1.514	12.0
P29 1 WEEK	1.45	1.46	1.374	1.428	11.2
P29 1 MO	1.334	1.351	1.219	1.301	9.9
P29 3 MO	1.604	1.585	1.578	1.589	12.8
P29 6 MO	1.254	1.238	1.239	1.244	9.3
P30 PRE	1.475	1.452	1.499	1.475	11.6
P30 1 DAY	1.403	1.365	1.395	1.388	10.8
P30 1WEEK	1.464	1.484	1.574	1.507	12.0
P30 1 MO	1.37	1.4	1.397	1.389	10.8
P30 3 MO	0.911	0.912	0.957	0.927	6.2
P30 6 MO	1.263	1.21	1.341	1.271	9.6

ELISA - LASIK P31 - P33 100 ng **4/18/13**

pLAC 3/20/13 = 324 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes
Read at 415 nm

AT	pLAC (ng)	abs 1	abs 2	abs 3	average
	16	1.497	1.486	1.483	1.489
	14	1.276	1.31	1.267	1.284
	12	0.966	0.961	0.989	0.972
	10	0.817	0.821	0.789	0.809
	8	0.575	0.561	0.557	
	6	0.477	0.432	0.432	0.447
	4	0.305	0.3	0.321	0.309
	2	0.211	0.219	0.215	0.215
	0	0.175	0.175	0.169	



Tear	abs 1	abs 2	abs 3	average	% lacritin
P31 PREOP	1.22	1.274	1.295	1.263	14.3
P31 1 DAY	0.682	0.667	0.641	0.663	7.8
P31 1 WK	1.415	1.42	1.393	1.409	15.9
P31 1 MO	1.736	1.752	1.75	1.746	19.5
P31 3 MO	1.257	1.237	1.202	1.232	13.9
P33 PREOP	1.651	1.63	1.575	1.619	18.1
P33 1 DAY	1.205	1.207	1.169	1.194	13.5
P33 1 WK	1.711	1.739	1.731	1.727	19.3
P33 1 MO	1.642	1.581	1.47	1.564	17.6
P33 3 MO	1.274	1.275	1.218	1.256	14.2

ELISA - LASIK P31 - P33 100 ng **4/18/13**

pLAC 3/20/13 = 324 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

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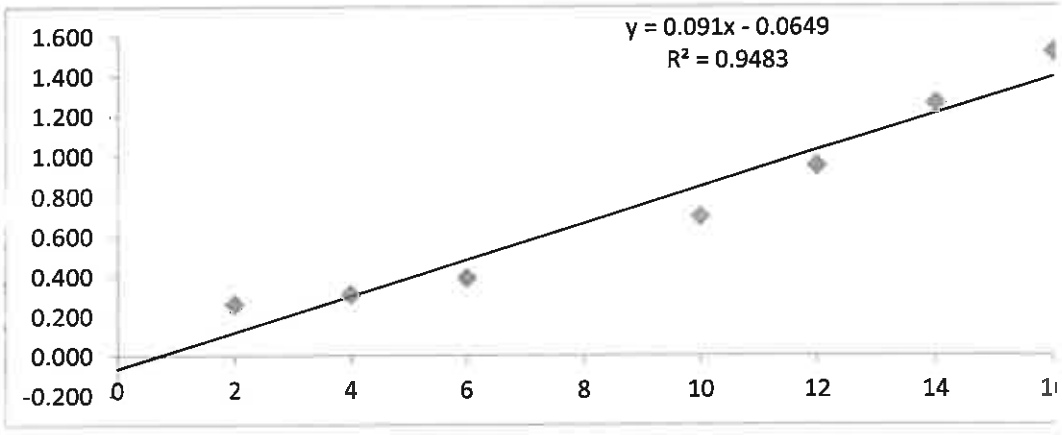
pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.444	1.532	1.576	1.517
14	1.246	1.262	1.273	1.260
12	0.943	0.939	0.958	0.947
10	0.682	0.703	0.704	0.696
8	0.479	0.49	0.492	
6	0.358	0.369	0.438	0.388
4	0.309	0.301	0.31	0.307
2	0.24	0.248	0.284	0.257
0	0.204	0.213	0.215	

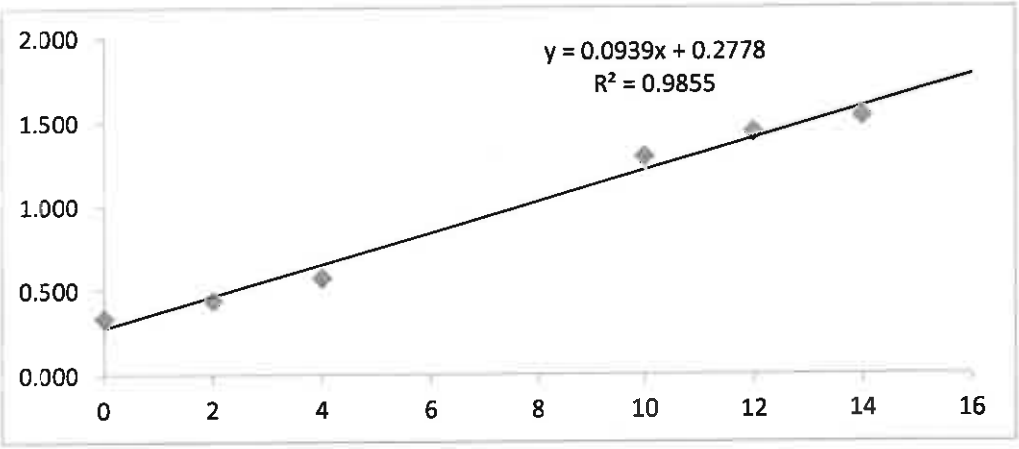


Tear	abs 1	abs 2	abs 3	average	% lacritin
P31 PREOP	1.287	1.336	1.481	1.368	15.7
P31 1 DAY	0.693	0.695	0.734	0.707	8.5
P31 1 WK	1.461	1.443	1.466	1.457	16.7
P31 1 MO	1.894	1.827	1.841	1.854	21.1
P31 3 MO	1.285	1.337	1.269	1.297	15.0
P33 PREOP	1.7	1.723	1.723	1.715	19.6
P33 1 DAY	1.288	1.304	1.293	1.295	14.9
P33 1 WK	1.794	1.841	1.794	1.810	20.6
P33 1 MO	1.602	1.636	1.681	1.640	18.7
P33 3 MO	1.405	1.426	1.425	1.419	16.3

ELISA - LASIK P31 - P58 PRE-3MO 100 ng **8/20/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.491	1.629	1.663	
14	1.502	1.545	1.547	1.531
12	1.444	1.449	1.433	1.442
10	1.309	1.271	1.302	1.294
6	0.623	0.718	0.653	
4	0.471	0.61	0.631	0.571
2	0.441	0.417	0.459	0.439
0	0.331	0.323	0.342	0.332

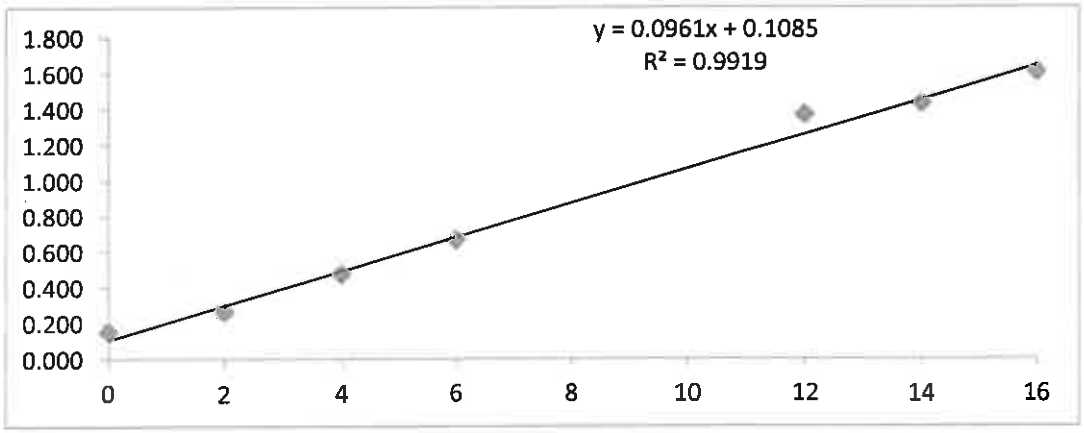


Tear	abs 1	abs 2	abs 3	average	% lacritin
P31 PRE	1.351	1.345	1.293	1.330	11.2
P31 1 DAY	1.006	0.977	0.967	0.983	7.5
P31 1 WEEK	0.823	0.825	1.002	0.883	6.4
P31 1 MO	1.348	1.35	1.269	1.322	11.1
P31 3 MO	0.967	0.974	0.949	0.963	7.3
P31 6 MO	0.733	0.749	0.752	0.745	5.0
P58 PRE	1.244	1.256	1.287	1.262	10.5
P58 1 DAY	0.466	0.467	0.479	0.471	2.1
P58 1 WEEK	1.36	1.236	1.472	1.356	11.5
P58 1 MO	1.305	1.294	1.353	1.317	11.1
P58 3 MO	0.976	1.086	1.125	1.062	8.4

ELISA - LASIK P31 - P58 PRE-3MO 100 ng **8/20/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.644	1.589	1.584	1.606
14	1.429	1.441	1.422	1.431
12	1.374	1.39	1.34	1.368
10	1.352	1.367	1.328	
6	0.655	0.663	0.682	0.667
4	0.469	0.467	0.479	0.472
2	0.256	0.261	0.258	0.259
0	0.152	0.145	0.152	0.150

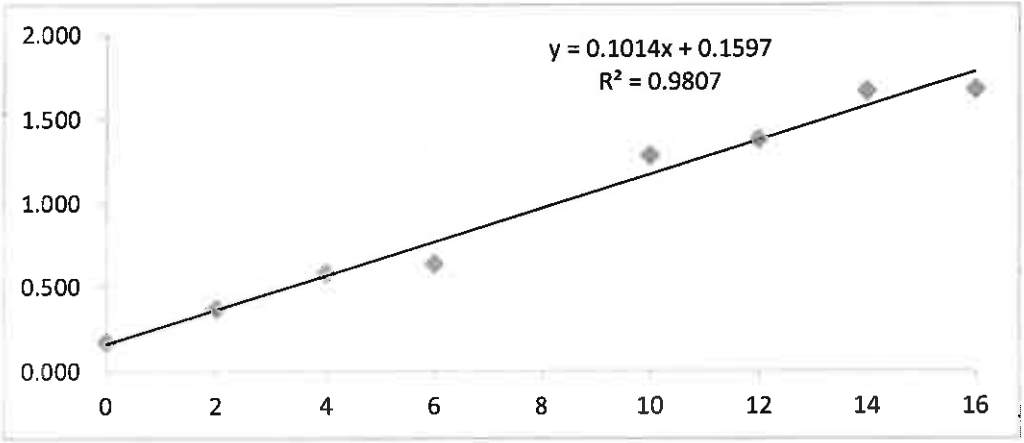


Tear	abs 1	abs 2	abs 3	average	% lacritin
P31 PRE	1.234	1.201	1.191	1.209	11.4
P31 1 DAY	1.1	1.02	0.996	1.039	9.7
P31 1 WEEK	0.906	0.885	0.839	0.877	8.0
P31 1 MO	1.225	1.294	1.254	1.258	12.0
P31 3 MO	0.813	0.814	0.841	0.823	7.4
P31 6 MO	0.569	0.56	0.564	0.564	4.7
P58 PRE	1.114	1.126	1.149	1.130	10.6
P58 1 DAY	0.284	0.293	0.287	0.288	1.9
P58 1 WEEK	1.269	1.307	1.275	1.284	12.2
P58 1 MO	1.203	1.247	1.262	1.237	11.7
P58 3 MO	1.021	1.007	0.995	1.008	9.4

ELISA - LASIK P35- P38 100 ng **7/17/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.669	1.697	1.667	1.678
14	1.621	1.684	1.691	1.665
12	1.319	1.469	1.35	1.379
10	1.205	1.423	1.227	1.285
6	0.628	0.641	0.639	0.636
4	0.579	0.57	0.597	0.582
2	0.376	0.361	0.37	0.369
0	0.163	0.167	0.18	0.170

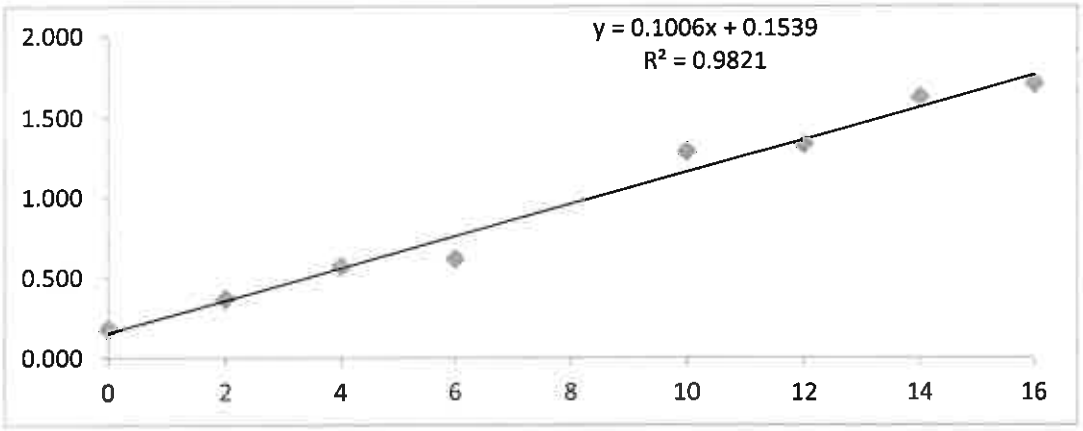


Tear	abs 1	abs 2	abs 3	average	% lacritin
P35 PRE	1.514	1.475	1.449	1.479	13.0
P35 1 DAY	1.762	1.659	1.688	1.703	15.2
P35 1 WK	1.348	1.338	1.283	1.323	11.5
P35 1 MO	1.297	1.298	1.336	1.310	11.3
P35 3 MO	1.435	1.267	1.272	1.325	11.5
P35 6 MO	1.667	1.597	1.651	1.638	14.6
P38 PRE	1.22	1.343	1.201	1.255	10.8
P38 1 DAY	0.994	0.965	0.928	0.962	7.9
P38 1 WEEK	1.217	1.336	1.355	1.303	11.3
P38 1 MO	1.507	1.573	1.484	1.521	13.4
P38 3 MO	1.606	1.562	1.562	1.577	14.0
P38 6 MO	1.37	1.363	1.357	1.363	11.9

ELISA - LASIK P35- P38 100 ng **7/17/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.737	1.684	1.693	1.705
14	1.635	1.632	1.594	1.620
12	1.315	1.338	1.341	1.331
10	1.266	1.329	1.258	1.284
6	0.618	0.606	0.622	0.615
4	0.558	0.574	0.571	0.568
2	0.362	0.369	0.367	0.366
0	0.177	0.177	0.18	0.178

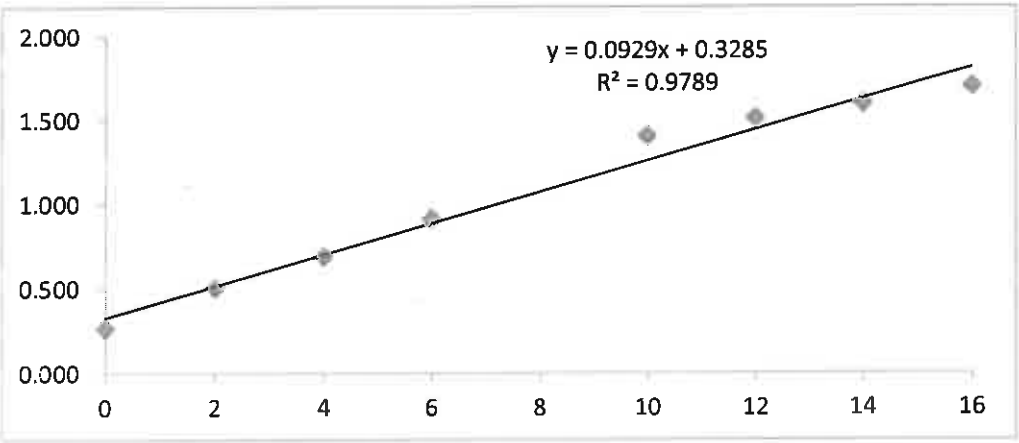


Tear	abs 1	abs 2	abs 3	average	% lacritin
P35 PRE	1.575	1.613	1.822	1.670	15.1
P35 1 DAY	1.822	1.746	1.655	1.741	15.8
P35 1 WK	1.388	1.408	1.439	1.412	12.5
P35 1 MO	1.289	1.288	1.34	1.306	11.4
P35 3 MO	1.367	1.347	1.346	1.353	11.9
P35 6 MO	1.683	1.726	1.677	1.695	15.3
P38 PRE	0.872	0.879	0.888	0.880	7.2
P38 1 DAY	0.843	0.828	0.777	0.816	6.6
P38 1 WEEK	1.322	1.314	1.333	1.323	11.6
P38 1 MO	1.511	1.546	1.562	1.540	13.8
P38 3 MO	1.611	1.612	1.59	1.604	14.4
P38 6 MO	1.432	1.509	1.423	1.455	12.9

ELISA - LASIK P39 - P41 100 ng **8/01/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.663	1.692	1.742	1.699
14	1.606	1.621	1.565	1.597
12	1.539	1.562	1.425	1.509
10	1.489	1.382	1.338	1.403
6	0.912	1.016	0.806	0.911
4	0.674	0.761	0.628	0.688
2	0.569	0.528	0.411	0.503
0	0.307	0.304	0.186	0.266

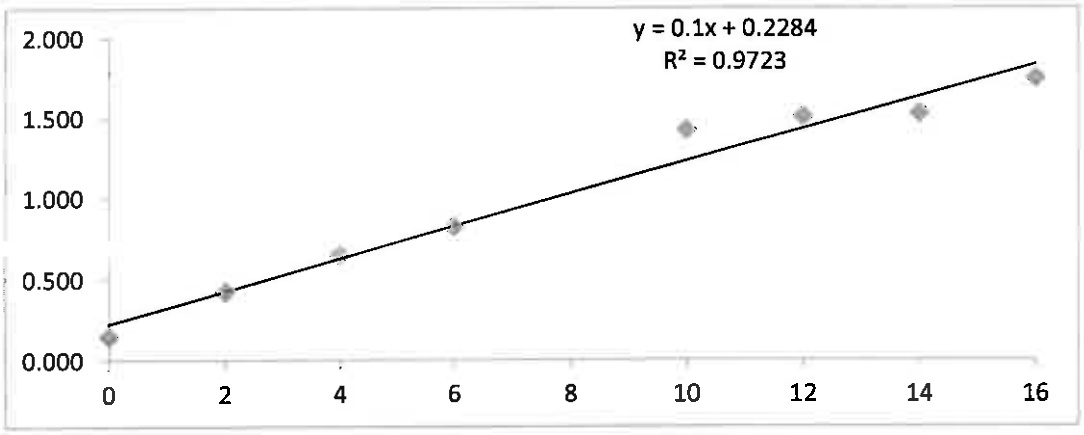


Tear	abs 1	abs 2	abs 3	average	% lacritin
P39 PREOP	0.955	1.034	1.062	1.017	7.4
P39 1 DAY	1.096	1.055	1.156	1.102	8.3
P39 1 WK	1.205	1.156	1.133	1.165	9.0
P39 1 MO	1.056	1.057	1.181	1.098	8.3
P39 3 MO	1.061	1.041	1.034	1.045	7.7
P39 6 MO	1.28	1.128	1.161	1.190	9.3
P41 PREOP	1.345	1.361	1.233	1.313	10.6
P41 1 DAY	1.02	0.879	0.937	0.945	6.6
P41 1 WK	1.544	1.349	1.522	1.472	12.3
P41 1 MO	1.444	1.655	1.601	1.567	13.3
P41 3 MO	1.147	1.155	1.14	1.147	8.8
P41 6 MO	0.987	1.061	1.077	1.042	7.7

ELISA - LASIK P39-41 100 ng **8/01/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.695	1.79	1.743	1.743
14	1.527	1.539	1.505	1.524
12	1.463	1.536	1.515	1.505
10	1.382	1.463	1.412	1.419
6	0.776	0.836	0.851	0.821
4	0.614	0.67	0.675	0.653
2	0.41	0.432	0.41	0.421
0	0.144	0.143	0.145	0.144

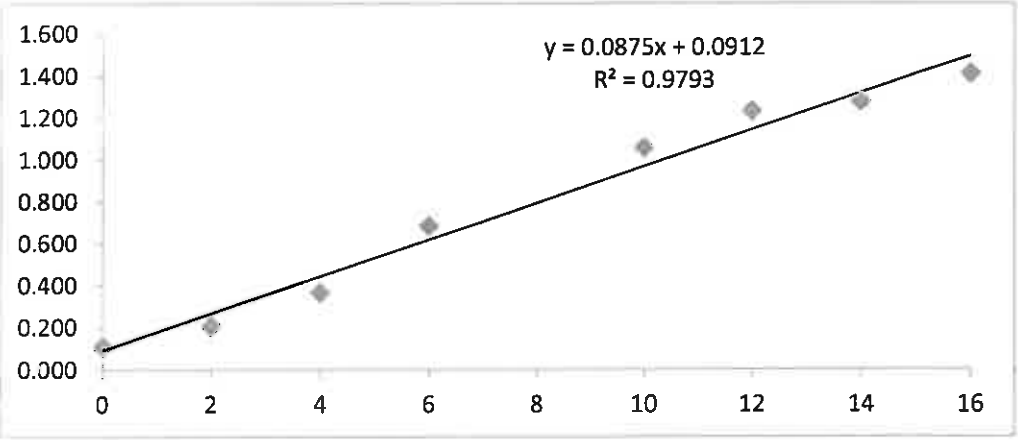


Tear	abs 1	abs 2	abs 3	average	% lacritin
P39 PREOP	1.029	0.997	1.109	1.045	8.2
P39 1 DAY	1.178	1.086	1.095	1.120	8.9
P39 1 WK	1.247	1.197	1.243	1.229	10.0
P39 1 MO	1.132	1.245	1.086	1.154	9.3
P39 3 MO	1.064	1.068	1.07	1.067	8.4
P39 6 MO	1.219	1.211	1.22	1.217	9.9
P41 PREOP	1.315	1.348	1.29	1.318	10.9
P41 1 DAY	0.885	0.855	0.836	0.859	6.3
P41 1 WK	1.437	1.47	1.428	1.445	12.2
P41 1 MO	1.556	1.623	1.543	1.574	13.5
P41 3 MO	1.208	1.217	1.231	1.219	9.9
P41 6 MO	1.066	1.067	1.091	1.075	8.5

ELISA - LASIK P42 - P43 100 ng **7/25/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.38	1.408	1.437	1.408
14	1.266	1.273	1.278	1.272
12	1.212	1.254	1.226	1.231
10	1.023	1.08	1.061	1.055
6	0.672	0.682	0.693	0.682
4	0.37	0.362	0.359	0.364
2	0.204	0.205	0.212	0.207
0	0.104	0.123	0.101	0.109



Tear	abs 1	abs 2	abs 3	average	% lacritin
P42 PREOP	1.083	1.077	1.073	1.078	11.3
P42 1 DAY	1.35	1.405	1.42	1.392	14.9
P42 1 WK	0.94	0.959	0.968	0.956	9.9
P42 1 MO	1.284	1.312	1.291	1.296	13.8
P42 3 MO	1.065	1.071	1.122	1.086	11.4
P42 6 MO	1.081	1.044	1.048	1.058	11.0
P43 PREOP	1.119	1.126	1.087	1.111	11.7
P43 1 DAY	0.894	0.909	0.873	0.892	9.2
P43 1 WK	0.897	0.894	0.886	0.892	9.2
P43 1 MO	1.042	1.099	1.029	1.057	11.0
P43 3 MO	0.691	0.734	0.767	0.731	7.3
P43 6 MO	0.587	0.613	0.594	0.598	5.8

ELISA - LASIK P42 - P43 100 ng **7/25/13**

pLAC 5/29/13 = 321 ug/mL

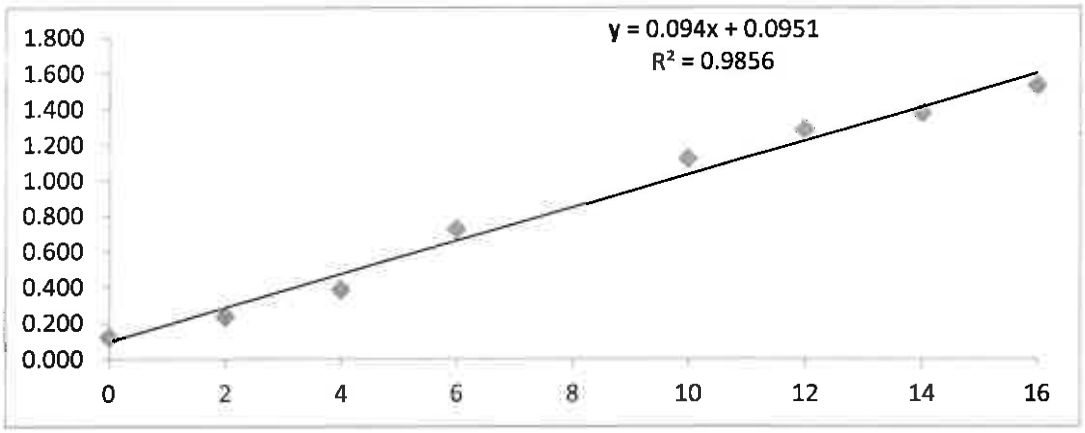
Blocking buffer = 1% BSA

Primary antibody = 1:400 Final Bleed 5529

Secondary antibody = 1:800 goat anti rabbit HRP

Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.516	1.529	1.551	1.532
14	1.364	1.386	1.388	1.379
12	1.258	1.279	1.31	1.282
10	1.115	1.132	1.117	1.121
6	0.696	0.72	0.755	0.724
4	0.374	0.386	0.393	0.384
2	0.228	0.241	0.236	0.235
0	0.118	0.122	0.117	0.119

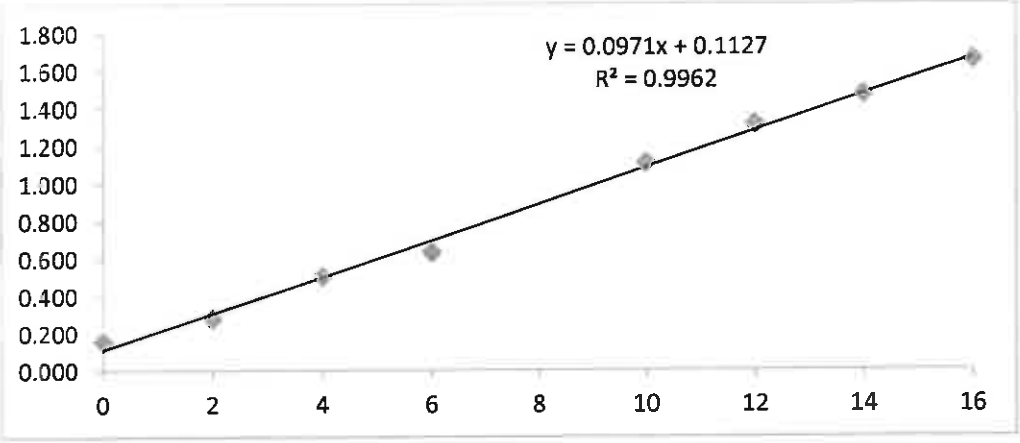


Tear	abs 1	abs 2	abs 3	average	% lacritin
P42 PREOP	1.164	1.169	1.142	1.158	11.3
P42 1 DAY	1.431	1.475	1.398	1.435	14.3
P42 1 WK	1.049	1.037	0.955	1.014	9.8
P42 1 MO	1.341	1.449	1.304	1.365	13.5
P42 3 MO	1.232	1.228	1.265	1.242	12.2
P42 6 MO	1.141	1.141	1.119	1.134	11.0
P43 PREOP	1.333	1.341	1.244	1.306	12.9
P43 1 DAY	0.945	0.957	0.926	0.943	9.0
P43 1 WK	0.981	0.982	0.914	0.959	9.2
P43 1 MO	1.15	1.14	0.967	1.086	10.5
P43 3 MO	0.726	0.837	0.832	0.798	7.5
P43 6 MO	0.625	0.618	0.629	0.624	5.6

ELISA - LASIK P44 - P46 100 ng **7/30/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.672	1.672	1.621	1.655
14	1.466	1.472	1.469	1.469
12	1.318	1.337	1.282	1.312
10	1.118	1.114	1.09	1.107
6	0.629	0.651	0.62	0.633
4	0.524	0.501	0.49	0.505
2	0.273	0.295	0.266	0.278
0	0.157	0.16	0.154	0.157



Tear	abs 1	abs 2	abs 3	average	% lacritin
P44 PREOP	1.295	1.258	1.278	1.277	12.0
P44 1 DAY	0.954	0.949	0.949	0.951	8.6
P44 1 WK	1.295	1.321	1.31	1.309	12.3
P44 1 MO	1.423	1.419	1.43	1.424	13.5
P44 3 MO	1.294	1.268	1.391	1.318	12.4
P44 6 MO	1.222	1.268	1.242	1.244	11.7
P46 PREOP	1.161	1.161	1.195	1.172	10.9
P46 1 DAY	1.103	1.125	1.135	1.121	10.4
P46 1 WK	1.481	1.601	1.558	1.547	14.8
P46 1 MO	1.466	1.654	1.664	1.595	15.3
P46 3 MO	1.475	1.519	1.494	1.496	14.2
P46 6 MO	1.363	1.386	1.5	1.416	13.4

ELISA - LASIK P44-46 100 ng **7/30/13**

pLAC 5/29/13 = 321 ug/mL

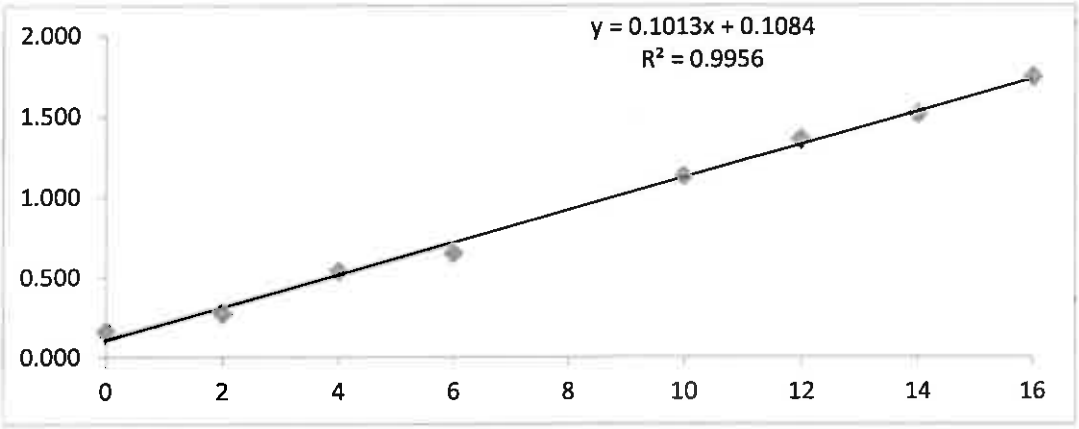
Blocking buffer = 1% BSA

Primary antibody = 1:400 Final Bleed 5529

Secondary antibody = 1:800 goat anti rabbit HRP

Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.763	1.734	1.718	1.738
14	1.536	1.514	1.482	1.511
12	1.341	1.373	1.355	1.356
10	1.109	1.139	1.136	1.128
6	0.632	0.659	0.652	0.648
4	0.554	0.529	0.526	0.536
2	0.268	0.274	0.277	0.271
0	0.158	0.16	0.162	0.160

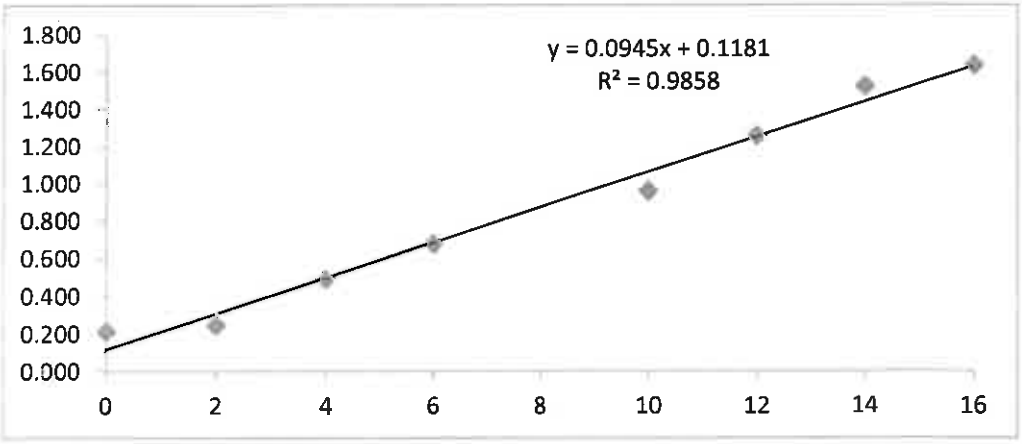


Tear	abs 1	abs 2	abs 3	average	% lacritin
P44 PREOP	1.353	1.402	1.336	1.364	12.4
P44 1 DAY	0.921	0.944	1.008	0.958	8.4
P44 1 WK	1.305	1.372	1.383	1.353	12.3
P44 1 MO	1.489	1.446	1.385	1.440	13.1
P44 3 MO	1.419	1.385	1.46	1.421	13.0
P44 6 MO	1.328	1.298	1.336	1.321	12.0
P46 PREOP	1.249	1.259	1.262	1.257	11.3
P46 1 DAY	1.156	1.214	1.161	1.177	10.5
P46 1 WK	1.573	1.641	1.689	1.634	15.1
P46 1 MO	0.447	1.665	1.747	1.286	11.6
P46 3 MO	1.637	1.579	1.556	1.591	14.6
P46 6 MO	1.482	1.523	1.635	1.547	14.2

ELISA - LASIK P47 - P49 100 ng **7/31/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.638	1.646	1.619	1.634
14	1.498	1.506	1.564	1.523
12	1.233	1.28	1.255	1.256
10	0.977	0.937	0.958	0.957
6	0.709	0.666	0.655	0.677
4	0.488	0.498	0.48	0.489
2	0.242	0.244	0.245	0.244
0	0.283	0.203	0.152	0.213

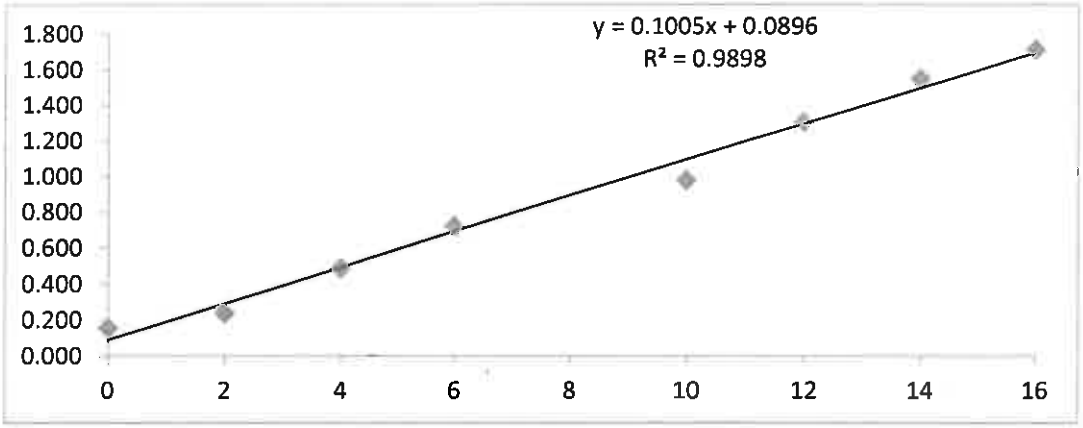


Tear	abs 1	abs 2	abs 3	average	% lacritin
P47 PREOP	1.12	1.1	1.112	1.111	10.5
P47 1 DAY	1.47	1.442	1.495	1.469	14.3
P47 1 WK	1.395	1.433	1.41	1.413	13.7
P47 1 MO	1.389	1.439	1.432	1.420	13.8
P47 3 MO	1.513	1.516	1.55	1.526	14.9
P47 6 MO	1.499	1.611	1.538	1.549	15.1
P49 PREOP	1.354	1.197	1.228	1.260	12.1
P49 1 DAY	0.421	0.242	0.247	0.303	2.0
P49 1 WK	0.59	1.335	1.346	1.090	10.3
P49 1 MO	0.282	1.198	1.139	0.873	8.0
P49 3 MO	1.129	1.178	1.169	1.159	11.0
P49 6 MO	1.388	1.382	1.399	1.390	13.5

ELISA - LASIK P47-49 100 ng **7/31/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.738	1.712	1.691	1.714
14	1.572	1.526	1.557	1.552
12	1.28	1.345	1.291	1.305
10	0.961	1.004	0.968	0.978
6	0.714	0.71	0.739	0.721
4	0.463	0.489	0.512	0.488
2	0.233	0.243	0.242	0.238
0	0.156	0.158	0.153	0.156

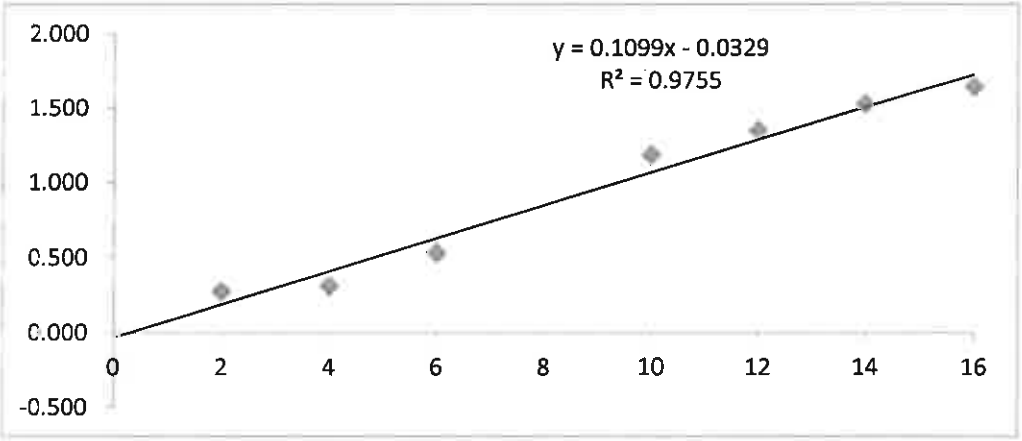


Tear	abs 1	abs 2	abs 3	average	% lacritin
P47 PREOP	1.248	1.199	1.175	1.207	11.1
P47 1 DAY	1.589	1.573	1.567	1.576	14.8
P47 1 WK	1.49	1.494	1.531	1.505	14.1
P47 1 MO	1.52	1.523	1.438	1.494	14.0
P47 3 MO	1.612	1.536	1.109	1.419	13.2
P47 6 MO	1.597	1.633	1.624	1.618	15.2
P49 PREOP	1.319	1.326	1.295	1.313	12.2
P49 1 DAY	0.239	0.245	0.232	0.239	1.5
P49 1 WK	1.227	1.364	1.286	1.292	12.0
P49 1 MO	1.176	1.222	1.185	1.194	11.0
P49 3 MO	1.248	1.286	1.272	1.269	11.7
P49 6 MO	1.437	1.563	1.496	1.499	14.0

ELISA - LASIK P50 - P51 100 ng **4/26/13**

pLAC 3/20/13 = 243 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

CS	pLAC (ng)	abs 1	abs 2	abs 3	average
	16	1.635	1.655	1.639	1.643
	14	1.527	1.522	1.524	1.524
	12	1.324	1.358	1.354	1.345
	10	1.191	1.174	1.18	1.182
	6	0.519	0.53	0.532	0.527
	4	0.335	0.294	0.299	0.309
	2	0.334	0.25	0.232	0.272
	0	0.203	0.187	0.19	



Tear	abs 1	abs 2	abs 3	average	% lacritin
P50 PREOP	1.03	1.063	1.107	1.067	10.0
P50 1 DAY	1.002	1.138	1.09	1.077	10.1
P50 1 WK	1.068	1.137	1.098	1.101	10.3
P50 1 MO	0.761	0.781	0.781	0.774	7.4
P50 3 MO	0.71	0.75	0.735	0.732	7.0
P50 6 MO	0.968	1.033	0.97	0.990	9.3
P51 PREOP	1.383	1.399	1.41	1.397	13.0
P51 1 DAY	1.26	1.311	1.298	1.290	12.1
P51 1 WK	1.449	1.476	1.485	1.470	13.7
P51 1 MO	1.269	1.271	1.258	1.266	11.8
P51 3 MO	1.398	1.389	1.546	1.444	13.5
P51 6 MO	1.391	1.297	1.316	1.335	12.5

ELISA - LASIK P50 - P51 100 ng **4/26/13**

pLAC 3/20/13 = 243 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

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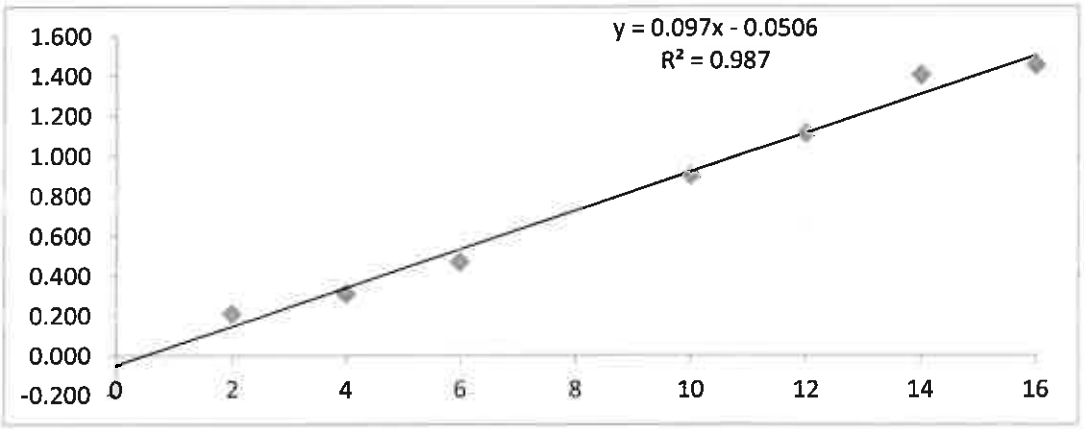
pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.438	1.466	1.46	1.455
14	1.315	1.416	1.477	1.403
12	0.967	1.181	1.189	1.112
10	0.772	0.947	0.987	0.902
6	0.385	0.505	0.514	0.468
4	0.321	0.294	0.311	0.309
2	0.203	0.205	0.217	0.208
0	0.343	0.339	0.317	

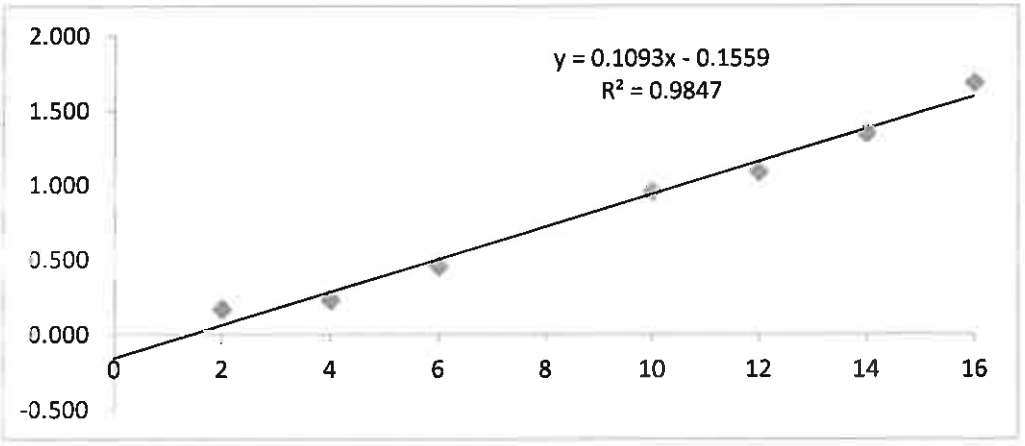


Tear	abs 1	abs 2	abs 3	average	% lacritin
P50 PREOP	1.298	1.244	1.255	1.266	13.6
P50 1 DAY	1.194	1.162	1.212	1.189	12.8
P50 1 WK	1.259	1.321	1.358	1.313	14.1
P50 1 MO	0.838	0.863	0.862	0.854	9.3
P50 3 MO	0.593	0.573	0.593	0.586	6.6
P50 6 MO	1.115	1.104	0.979	1.066	11.5
P51 PREOP	1.476	1.471	1.475	1.474	15.7
P51 1 DAY	1.453	1.475	1.401	1.443	15.4
P51 1 WK	1.715	1.611	1.553	1.626	17.3
P51 1 MO	1.261	1.248	1.36	1.290	13.8
P51 3 MO	1.351	1.38	1.396	1.376	14.7
P51 6 MO	1.431	1.434	1.392	1.419	15.2

ELISA - LASIK P55 - P56 100 ng **5/1/13**

pLAC 3/20/13 = 312 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

CS	pLAC (ng)	abs 1	abs 2	abs 3	average
	16	1.718	1.673	1.665	1.685
	14	1.381	1.333	1.317	1.344
	12	1.085	1.065	1.1	1.083
	10	0.978	0.948	0.934	0.953
	6	0.45	0.456	0.447	0.451
	4	0.233	0.221	0.221	0.225
	2	0.166	0.165	0.163	0.165
	0	0.127	0.137	0.14	



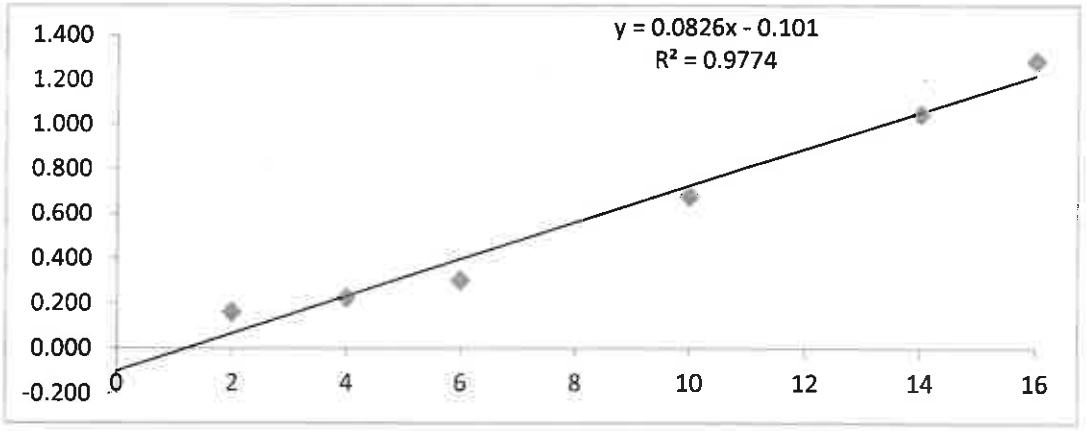
Tear	abs 1	abs 2	abs 3	average	% lacritin
P55 PREOP	1.113	1.124	1.144	1.127	11.7
P55 1 DAY	0.484	0.462	0.504	0.483	5.8
P55 1 WK	1.612	1.728	1.661	1.667	16.7
P55 1 MO	1.48	1.51	1.45	1.480	15.0
P55 3 MO	1.314	1.336	1.335	1.328	13.6
P55 6 MO	1.394	1.472	1.444	1.437	14.6
P56 PREOP	1.499	1.415	1.409	1.441	14.6
P56 1 DAY	0.934	0.937	0.952	0.941	10.0
P56 1 WK	1.549	1.514	1.64	1.568	15.8
P56 1 MO	1.428	1.454	1.472	1.451	14.7
P56 3 MO	1.44	1.44	1.455	1.445	14.6
P56 6 MO	1.497	1.47	1.628	1.532	15.4

ELISA - LASIK P55- P56 100 ng **5/1/13**

pLAC 3/20/13 = 312 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

AT

pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.244	1.318	1.293	1.285
14	1.072	1.005	1.06	1.046
12	0.694	0.686	0.694	
10	0.67	0.678	0.679	0.676
6	0.304	0.301	0.294	0.300
4	0.239	0.216	0.216	0.224
2	0.163	0.154	0.161	0.159
0	0.139	0.135	0.134	

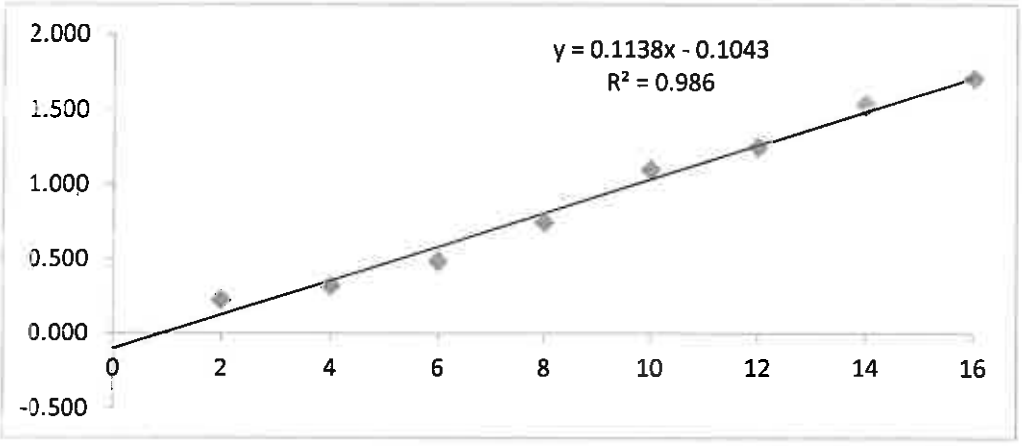


Tear	abs 1	abs 2	abs 3	average	% lacritin
P55 PREOP	1.129	1.144	1.101	1.125	14.8
P55 1 DAY	0.498	0.513	0.501	0.504	7.3
P55 1 WK	1.719	1.743	1.723	1.728	22.1
P55 1 MO	1.578	1.55	1.656	1.595	20.5
P55 3 MO	1.413	1.388	1.361	1.387	18.0
P55 6 MO	1.496	1.502	1.491	1.496	19.3
P56 PREOP	1.547	1.47	1.455	1.491	19.3
P56 1 DAY	0.945	0.93	0.917	0.931	12.5
P56 1 WK	1.581	1.599	1.59	1.590	20.5
P56 1 MO	1.419	1.443	1.441	1.434	18.6
P56 3 MO	1.477	1.437	1.452	1.455	18.8
P56 6 MO	1.489	1.528	1.497	1.505	19.4

ELISA - LASIK P57 - P58 100 ng **5/17/13**

pLAC 3/20/13 = 302 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.702	1.682	1.739	1.708
14	1.530	1.517	1.555	1.534
12	1.244	1.247	1.252	1.248
10	1.07	1.106	1.118	1.098
8	0.735	0.753	0.744	0.744
6	0.452	0.487	0.509	0.483
4	0.304	0.34	0.308	0.317
2	0.224	0.224	0.227	0.225
0	0.195	0.21	0.184	

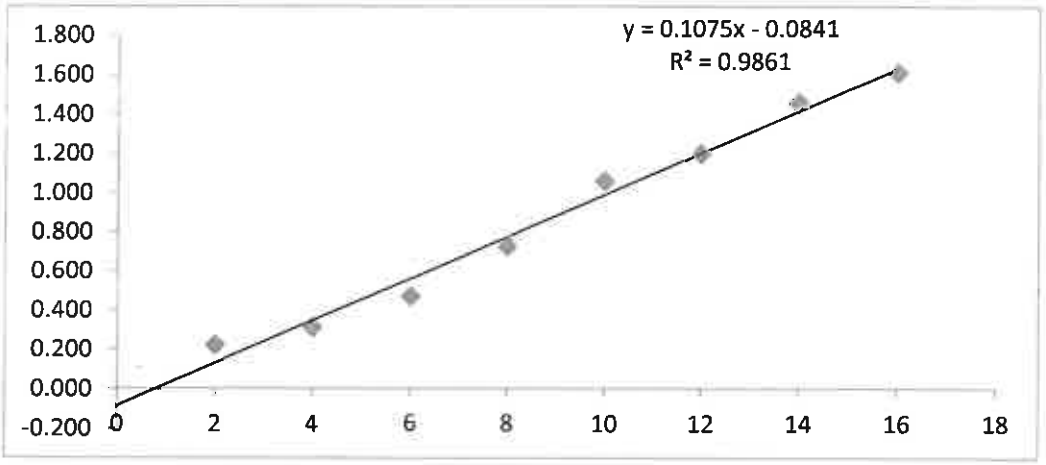


Tear	abs 1	abs 2	abs 3	average	% lacritin
P57 PREOP	1.098	1.158	1.108	1.121	10.8
P57 1 DAY	0.8	0.838	0.804	0.814	8.1
P57 1 WK	1.398	1.355	1.412	1.388	13.1
P57 1 MO	1.299	1.285	1.316	1.300	12.3
P57 3 MO	1.366	1.372	1.343	1.360	12.9
P57 6 MO	1.246	1.246	1.199	1.230	11.7
P58 PREOP	1.309	1.385	1.362	1.352	12.8
P58 1 DAY	0.339	0.345	0.333	0.339	3.9
P58 1 WK	1.23	1.303	1.333	1.289	12.2
P58 1 MO	1.213	1.202	1.204	1.206	11.5
P58 3 MO	1.106	1.084	1.098	1.096	10.5

ELISA - LASIK P57- P58 100 ng **5/17/13**

pLAC 3/20/13 = 302ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.587	1.595	1.662	1.615
14	1.464	1.454	1.469	1.462
12	1.196	1.209	1.203	1.203
10	1.102	1.042	1.042	1.062
8	0.753	0.718	0.706	0.726
6	0.483	0.467	0.461	0.470
4	0.308	0.304	0.315	0.309
2	0.222	0.222	0.217	0.220
0	0.193	0.186	0.178	

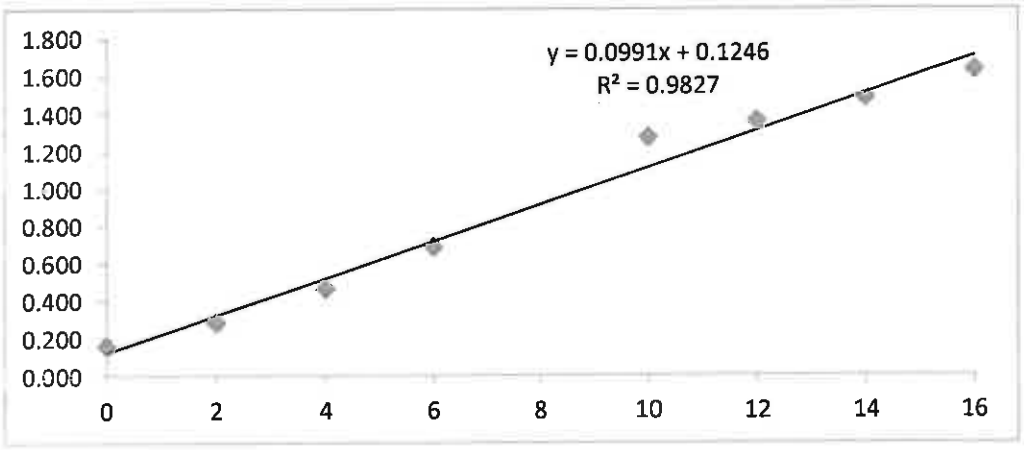


Tear	abs 1	abs 2	abs 3	average	% lacritin
P57 PREOP	1.103	1.137	1.127	1.122	11.2
P57 1 DAY	0.792	0.769	0.797	0.786	8.1
P57 1 WK	1.261	1.284	1.347	1.297	12.9
P57 1 MO	1.265	1.282	1.291	1.279	12.7
P57 3 MO	1.341	1.33	1.528	1.400	13.8
P57 6 MO	1.206	1.289	1.272	1.256	12.5
P58 PREOP	1.272	1.363	1.457	1.364	13.5
P58 1 DAY	0.329	0.334	0.391	0.351	4.1
P58 1 WK	1.057	1.19	1.464	1.237	12.3
P58 1 MO	1.134	1.174	1.204	1.171	11.7
P58 3 MO	1.007	1.002	1.023	1.011	10.2

ELISA - LASIK P59 - P61 100 ng **5/3/13**

pLAC 3/20/13 = 323 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

CS	pLAC (ng)	abs 1	abs 2	abs 3	average
	16	1.579	1.657	1.661	1.632
	14	1.456	1.495	1.498	1.483
	12	1.336	1.364	1.383	1.361
	10	1.27	1.273	1.279	1.274
	6	0.689	0.668	0.7	0.686
	4	0.465	0.452	0.472	0.463
	2	0.301	0.27	0.274	0.282
	0	0.156	0.16	0.155	0.157



Tear	abs 1	abs 2	abs 3	average	% lacritin
P59 PREOP	1.296	1.297	1.246	1.280	11.7
P59 1 DAY	1.362	1.265	1.352	1.326	12.1
P59 1 WK	1.239	1.327	1.257	1.274	11.6
P59 1 MO	1.605	1.695	1.718	1.673	15.6
P59 3 MO	1.017	1.016	1.037	1.023	9.1
P59 6 MO	1.402	1.371	1.402	1.392	12.8
P61 PREOP	1.52	1.552	1.556	1.543	14.3
P61 1 DAY	1.643	1.598	1.604	1.615	15.0
P61 1 WK	1.52	1.509	1.527	1.519	14.1
P61 1 MO	1.518	1.466	1.53	1.505	13.9
P61 3 MO	1.663	1.678	1.706	1.682	15.7
P61 6 MO	1.673	1.672	1.691	1.679	15.7

ELISA - LASIK P59- P61 100 ng **5/3/13**

pLAC 3/20/13 = 323 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

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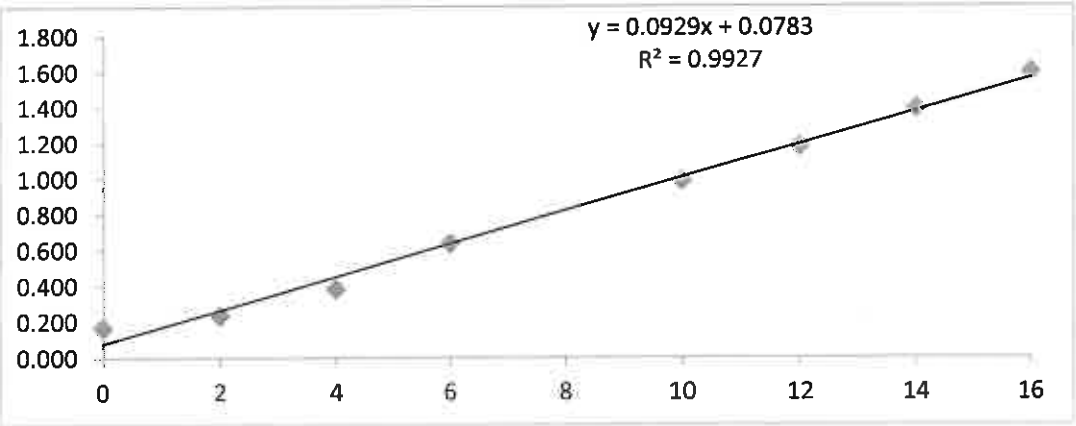
pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.53	1.622	1.638	1.597
14	1.376	1.398	1.406	1.393
12	1.169	1.168	1.197	1.178
10	0.966	0.987	1.001	0.985
6	0.626	0.622	0.658	0.635
4	0.388	0.385	0.372	0.382
2	0.235	0.223	0.242	0.233
0	0.167	0.159	0.173	0.166

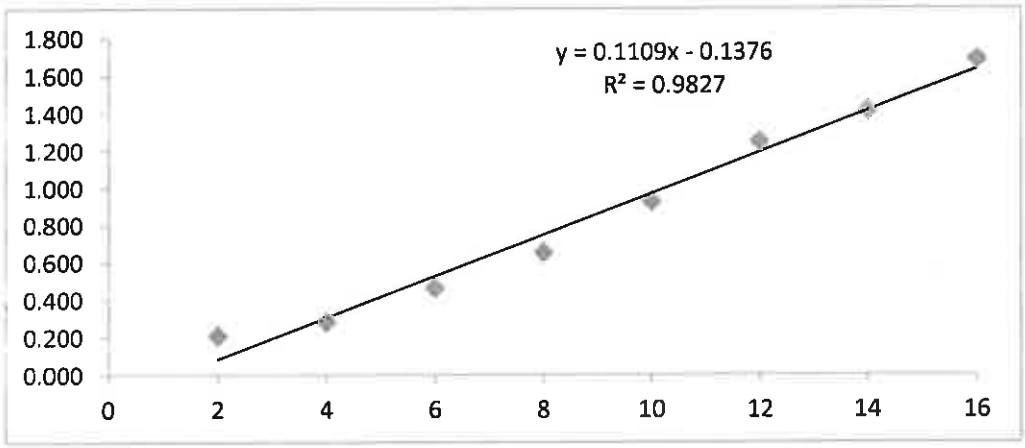


Tear	abs 1	abs 2	abs 3	average	% lacritin
P59 PREOP	1.222	1.269	1.219	1.237	12.5
P59 1 DAY	1.24	1.255	1.319	1.271	12.8
P59 1 WK	1.143	1.267	1.242	1.217	12.3
P59 1 MO	1.555	1.575	1.712	1.614	16.5
P59 3 MO	0.945	1.076	1.014	1.012	10.0
P59 6 MO	1.392	1.331	1.375	1.366	13.9
P61 PREOP	1.549	1.421	1.566	1.512	15.4
P61 1 DAY	1.629	1.656	1.632	1.639	16.8
P61 1 WK	1.546	1.523	1.591	1.553	15.9
P61 1 MO	1.426	1.453	1.541	1.473	15.0
P61 3 MO	1.677	1.687	1.686	1.683	17.3
P61 6 MO	1.656	1.692	1.733	1.694	17.4

ELISA - LASIK P62 - P63 Redo **5/9/13**

pLAC 3/20/13 = 275 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

CS	pLAC (ng)	abs 1	abs 2	abs 3	average
	16	1.711	1.697	1.651	1.686
	14	1.406	1.423	1.41	1.413
	12	1.219	1.23	1.298	1.249
	10	0.9	0.939	0.944	0.928
	8	0.647	0.663	0.651	0.654
	6	0.463	0.455	0.474	0.464
	4	0.28	0.282	0.287	0.283
	2	0.205	0.204	0.213	0.207
	0	0.167	0.165	0.168	0.167



Tear	abs 1	abs 2	abs 3	average	% lacritin
P62 PREOP	1.161	1.168	1.174	1.168	11.8
P62 1 DAY	0.371	0.348	0.35	0.356	4.5
P62 1 WK	1.323	1.399	1.387	1.370	13.6
P62 1 MO	0.939	0.924	0.955	0.939	9.7
P62 3 MO	0.743	0.712	0.713	0.723	7.8
P62 6 MO	1.052	1.168	1.011	1.077	11.0
P63 PREOP	1.108	1.119	1.044	1.090	11.1
P63 1 DAY	1.383	1.407	1.346	1.379	13.7
P63 1MO	1.19	1.205	1.217	1.204	12.1
P63 3 MO	1.383	1.455	1.423	1.420	14.0

ELISA - LASIK P62- P63 Redo **5/9/13**

pLAC 3/20/13 = 293 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

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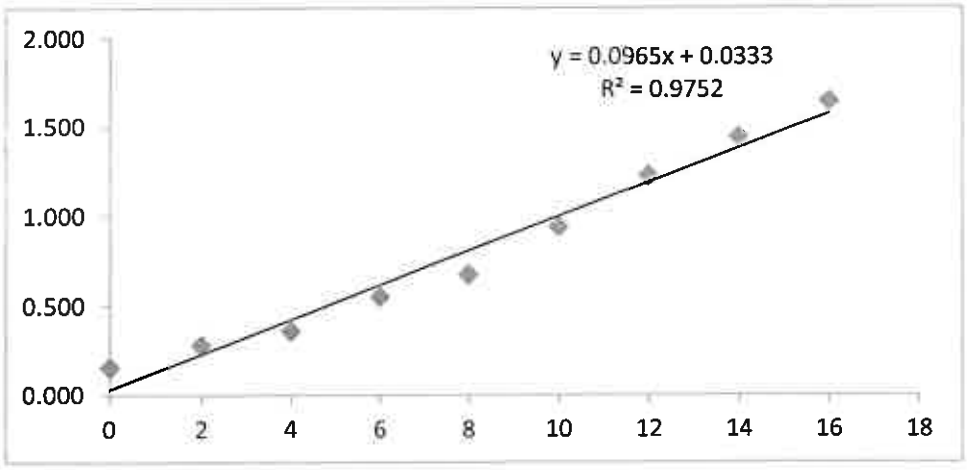
pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.636	1.63	1.664	1.643
14	1.403	1.459	1.46	1.441
12	1.208	1.219	1.25	1.226
10	0.923	0.94	0.948	0.937
8	0.651	0.67	0.685	0.669
6	0.534	0.515	0.589	0.546
4	0.331	0.37	0.365	0.355
2	0.27	0.264	0.298	0.277
0	0.159	0.149	0.153	0.154

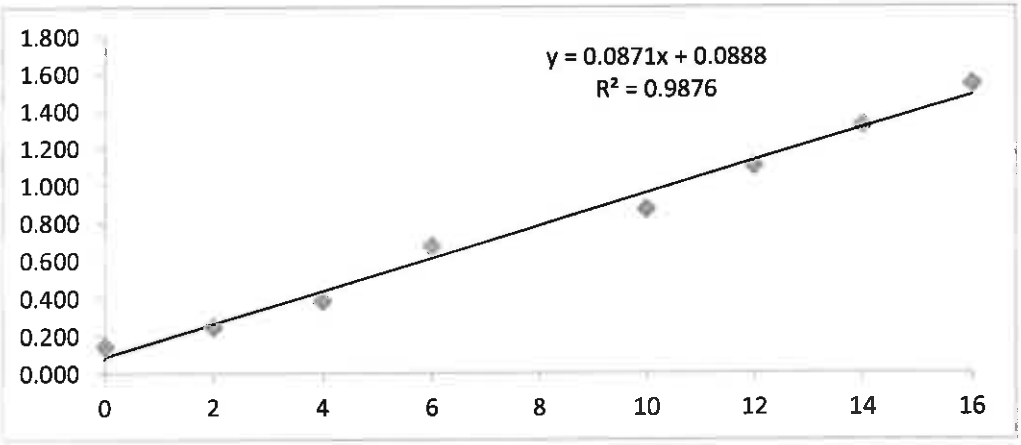


Tear	abs 1	abs 2	abs 3	average	% lacritin
P62 PREOP	1.12	1.067	1.073	1.087	10.9
P62 1 DAY	0.304	0.269	0.289	0.287	2.6
P62 1 WK	1.311	1.319	1.294	1.308	13.2
P62 1 MO	0.708	0.742	0.686	0.712	7.0
P62 3 MO	0.623	0.605	0.641	0.623	6.1
P62 6 MO	0.901	0.909	0.89	0.900	9.0
P63 PREOP	1.14-	1.151	1.171	1.161	11.7
P63 1 DAY	1.388	1.435	1.398	1.407	14.2
P63 1MO	1.202	1.22	1.172	1.198	12.1
P63 3 MO	1.412	1.46	1.433	1.435	14.5

ELISA - LASIK P64 - P65 100 ng **7/23/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.533	1.532	1.561	1.542
14	1.333	1.316	1.314	1.321
12	1.068	1.126	1.117	1.104
10	0.841	0.866	0.905	0.871
6	0.656	0.745	0.625	0.675
4	0.398	0.377	0.378	0.384
2	0.239	0.221	0.276	0.245
0	0.129	0.163	0.139	0.144

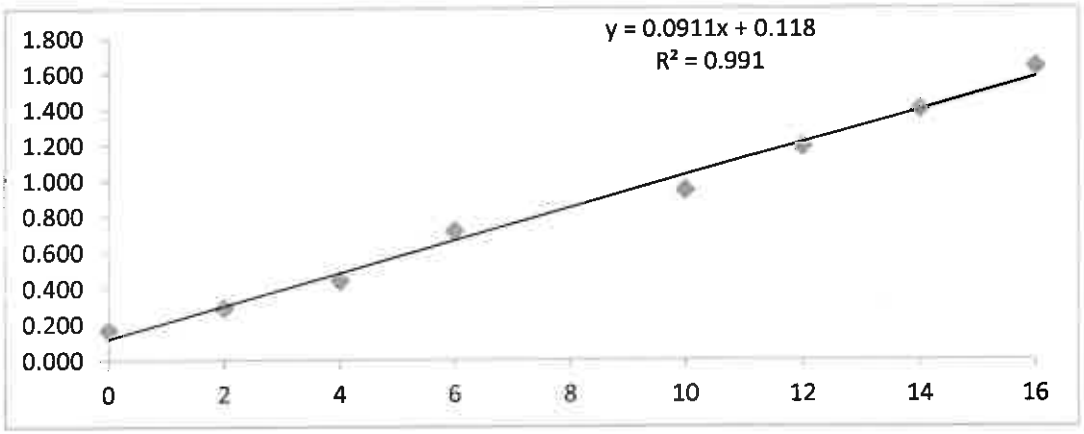


Tear	abs 1	abs 2	abs 3	average	% lacritin
P64 PREOP	0.776	0.78	0.814	0.790	8.1
P64 1 DAY	0.9	0.878	0.97	0.916	9.5
P64 1 WK	0.905	0.915	0.973	0.931	9.7
P64 1 MO	0.6	0.597	0.68	0.626	6.2
P64 3 MO	0.909	0.875	0.893	0.892	9.2
P64 6 MO	1.329	1.208	1.295	1.277	13.6
P65 PREOP	0.565	0.565	0.583	0.571	5.5
P65 1 DAY	0.404	0.395	0.405	0.401	3.6
P65 1 WK	0.409	0.425	0.512	0.449	4.1
P65 1 MO	0.591	0.637	0.599	0.609	6.0
P65 3 MO	0.576	0.482	0.541	0.533	5.1
P65 6 MO	0.78	0.77	0.798	0.783	8.0

ELISA - LASIK P64 - P65 100 ng **7/23/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.611	1.662	1.633	1.635
14	1.404	1.41	1.378	1.397
12	1.172	1.197	1.195	1.188
10	0.925	0.942	0.959	0.942
6	0.697	0.748	0.701	0.715
4	0.456	0.447	0.42	0.441
2	0.295	0.285	0.276	0.290
0	0.166	0.163	0.162	0.164

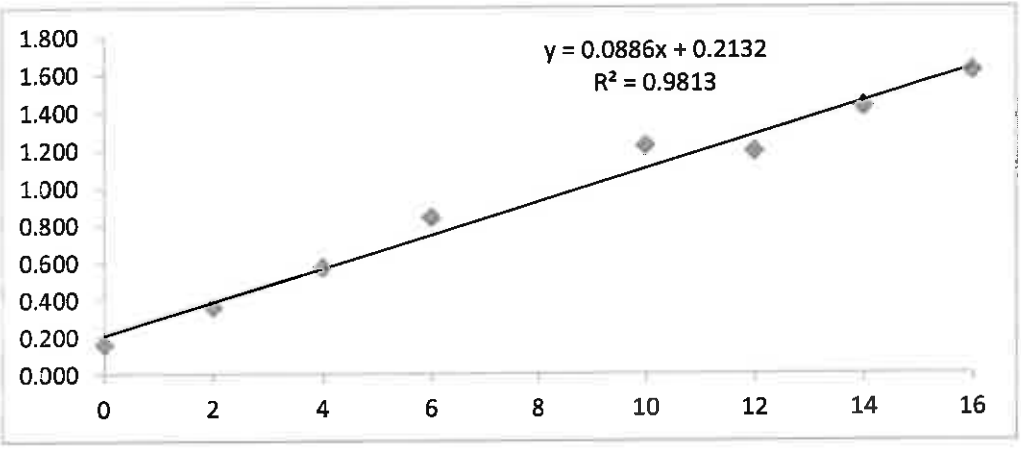


Tear	abs 1	abs 2	abs 3	average	% lacritin
P64 PREOP	0.86	0.841	0.814	0.838	7.9
P64 1 DAY	0.947	0.898	0.97	0.938	9.0
P64 1 WK	1.014	1.02	0.973	1.002	9.7
P64 1 MO	0.713	0.682	0.68	0.692	6.3
P64 3 MO	1.008	1	0.992	1.000	9.7
P64 6 MO	1.463	1.461	1.404	1.443	14.5
P65 PREOP	0.65	0.632	0.619	0.634	5.7
P65 1 DAY	0.443	0.417	0.432	0.431	3.4
P65 1 WK	0.492	0.486	0.471	0.483	4.0
P65 1 MO	0.664	0.658	0.627	0.650	5.8
P65 3 MO	0.686	0.609	0.627	0.641	5.7
P65 6 MO	0.897	0.841	0.916	0.885	8.4

ELISA - LASIK P67 - P68 100 ng **8/27/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.6	1.631	1.613	1.615
14	1.430	1.424	1.418	1.424
12	1.196	1.177	1.2	1.191
10	1.172	1.226	1.262	1.220
6	0.823	0.819	0.875	0.839
4	0.547	0.547	0.623	0.572
2	0.361	0.357	0.357	0.358
0	0.163	0.163	0.152	0.159

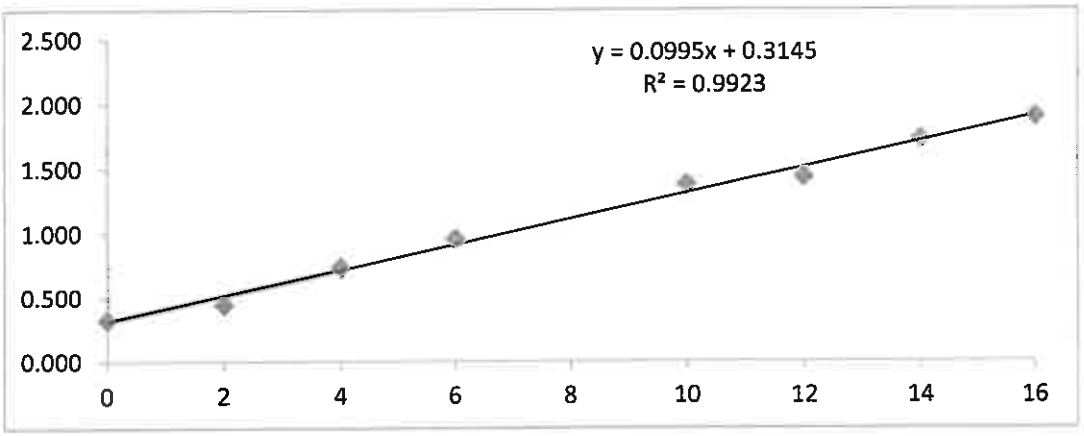


Tear	abs 1	abs 2	abs 3	average	% lacritin
P67 PRE	1.741	1.69	1.646	1.692	16.7
P67 1 DAY	1.53	1.49	1.485	1.502	14.5
P67 1 WEEK	1.787	1.757	1.678	1.741	17.2
P67 1 MO	1.864	1.756	1.748	1.789	17.8
P67 3 MO	1.681	1.707	1.708	1.699	16.8
P67 6 MO	1.684	1.718	1.681	1.694	16.7
P68 PRE	1.74	1.767	1.779	1.762	17.5
P68 1 DAY	1.653	1.701	1.679	1.678	16.5
P68 1 WEEK	1.783	1.972	1.853	1.869	18.7
P68 1 MO	1.703	1.941	1.869	1.838	18.3
P68 3 MO	1.809	1.778	1.829	1.805	18.0
P68 6 MO	1.614	1.765	1.753	1.711	16.9

ELISA - LASIK P67-P68 100 ng **8/27/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.857	1.936	1.894	1.896
14	1.744	1.708	1.714	1.722
12	1.429	1.446	1.418	1.431
10	1.487	1.291	1.346	1.375
6	0.906	0.885	1.082	0.958
4	0.633	0.728	0.848	0.736
2	0.372	0.512	0.494	0.442
0	0.333	0.36	0.276	0.323

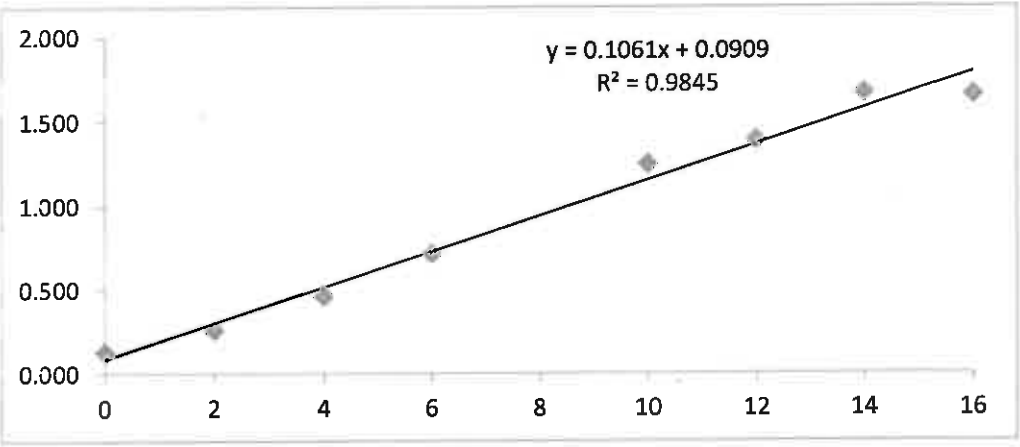


Tear	abs 1	abs 2	abs 3	average	% lacritin
P67 PRE	1.945	1.61	1.855	1.803	15.0
P67 1 DAY	1.701	1.576	1.698	1.658	13.5
P67 1 WEEK	1.802	1.87	1.908	1.860	15.5
P67 1 MO	2.009	1.957	1.63	1.865	15.6
P67 3 MO	1.9	1.94	1.691	1.844	15.4
P67 6 MO	1.847	1.988	1.965	1.933	16.3
P68 PRE	1.929	1.946	1.834	1.903	16.0
P68 1 DAY	1.843	1.849	1.848	1.847	15.4
P68 1 WEEK	2.079	2.105	2.055	2.080	17.7
P68 1 MO	1.897	2.004	1.879	1.927	16.2
P68 3 MO	1.808	1.89	1.972	1.890	15.8
P68 6 MO	1.641	1.713	1.674	1.676	13.7

ELISA - LASIK P69PRE-3MO - P73PRE-3MO 100 ng **8/15/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.677	1.648	1.64	1.655
14	1.682	1.656	1.67	1.669
12	1.419	1.392	1.359	1.390
10	1.265	1.22	1.253	1.246
6	0.714	0.71	0.721	0.715
4	0.484	0.438	0.456	0.459
2	0.258	0.264	0.251	0.258
0	0.129	0.127	0.127	0.128

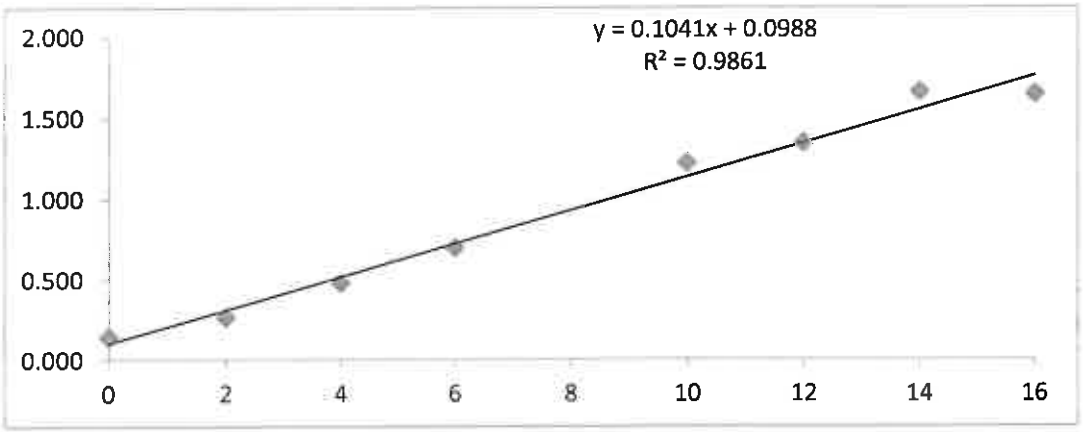


Tear	abs 1	abs 2	abs 3	average	% lacritin
P69 PRE	1.586	1.539	1.557	1.561	13.9
P69 1 DAY	1.515	1.516	1.445	1.492	13.2
P69 1 WEEK	1.842	1.757	1.903	1.834	16.4
P69 1 MO	1.514	1.519	1.614	1.549	13.7
P693MO	1.503	1.523	1.478	1.501	13.3
P73PRE	1.333	1.36	1.36	1.351	11.9
P73 1 DAY	1.151	1.186	1.227	1.188	10.3
P73 1 WEEK	1.562	1.615	1.64	1.606	14.3
P73 1 MO	1.22	1.233	1.273	1.242	10.8
P73 3 MO	1.347	1.514	1.487	1.449	12.8

ELISA - LASIK P69PRE-3MO - P73PRE-3MO 100 ng **8/15/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.65	1.66	1.635	1.648
14	1.657	1.663	1.664	1.661
12	1.318	1.37	1.356	1.348
10	1.232	1.211	1.22	1.221
6	0.697	0.69	0.706	0.698
4	0.473	0.462	0.499	0.478
2	0.266	0.259	0.266	0.263
0	0.143	0.136	0.138	0.139

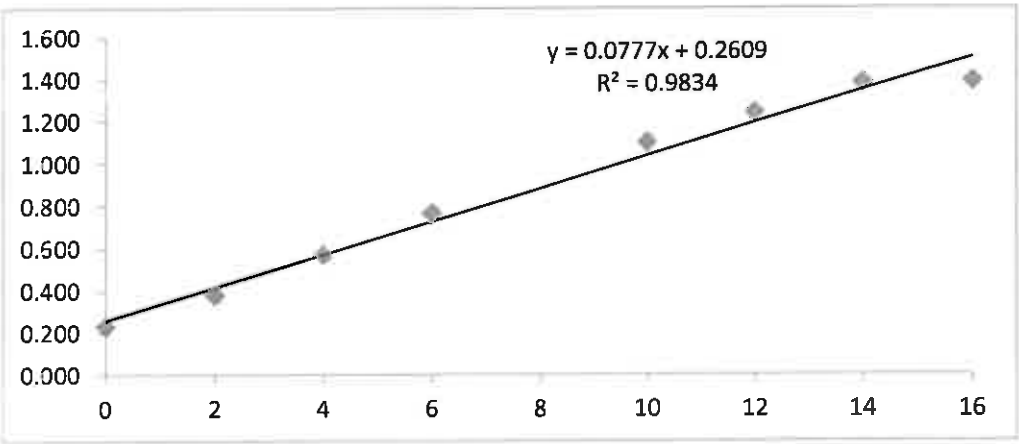


Tear	abs 1	abs 2	abs 3	average	% lacritin
P69 PRE	1.555	1.403	1.564	1.507	13.5
P69 1 DAY	1.534	1.456	1.386	1.459	13.1
P69 1 WEEK	1.822	1.731	1.88	1.811	16.4
P69 1 MO	1.504	1.447	1.389	1.447	12.9
P693MO	1.426	1.479	1.499	1.468	13.2
P73PRE	1.336	1.389	1.376	1.367	12.2
P73 1 DAY	1.193	1.204	1.189	1.195	10.5
P73 1 WEEK	1.512	1.569	1.55	1.544	13.9
P73 1 MO	1.157	1.326	1.167	1.217	10.7
P73 3 MO	1.343	1.501	1.461	1.435	12.8

ELISA - LASIK P74 PRE-3MO - P75 PRE-3MO 100 ng **8/22/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.372	1.405	1.389	1.389
14	1.276	1.453	1.418	1.382
12	1.214	1.35	1.159	1.241
10	1.085	1.135	1.079	1.100
6	0.741	0.785	0.771	0.766
4	0.589	0.547	0.576	0.571
2	0.372	0.347	0.415	0.378
0	0.304	0.198	0.191	0.231

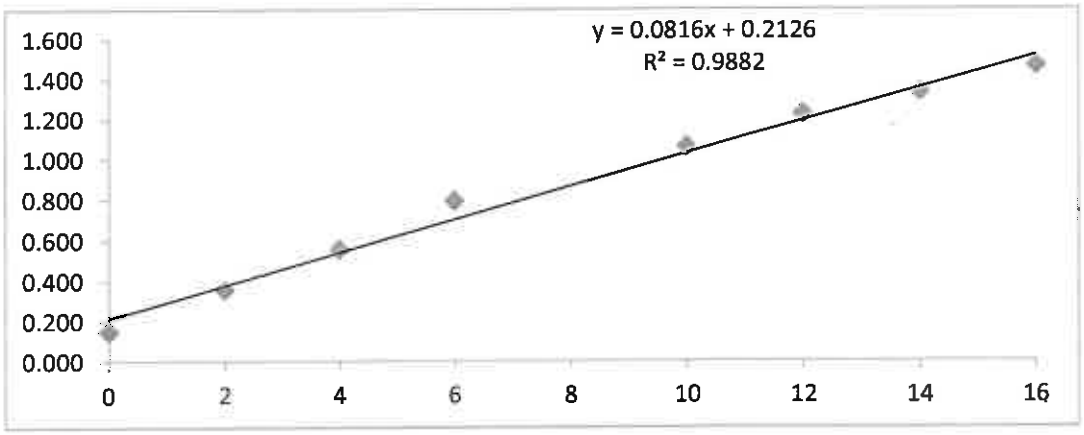


Tear	abs 1	abs 2	abs 3	average	% lacritin
P74 PRE	1.705	1.672	1.514	1.630	17.6
P74 1 DAY	1.701	1.624	1.729	1.685	18.3
P74 1 WEEK	1.704	1.639	1.777	1.707	18.6
P74 1 MO	1.722	1.564	1.396	1.561	16.7
P74 3 MO	1.646	1.709	1.677	1.677	18.2
P75 PRE	1.529	1.461	1.482	1.491	15.8
P751 DAY	1.839	1.732	1.719	1.763	19.3
P75 1 WEEK	1.82	1.885	1.763	1.823	20.1
P75 1 MO	1.826	1.827	1.838	1.830	20.2
P75 3 MO	1.668	1.892	1.884	1.815	20.0

ELISA - LASIK P74 PRE-3MO - P75 PRE-3MO 100 ng **8/22/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.432	1.476	1.476	1.461
14	1.323	1.326	1.346	1.332
12	1.221	1.248	1.202	1.224
10	1.029	1.077	1.074	1.060
6	0.759	0.801	0.819	0.793
4	0.568	0.542	0.556	0.555
2	0.344	0.359	0.359	0.354
0	0.143	0.152	0.144	0.146

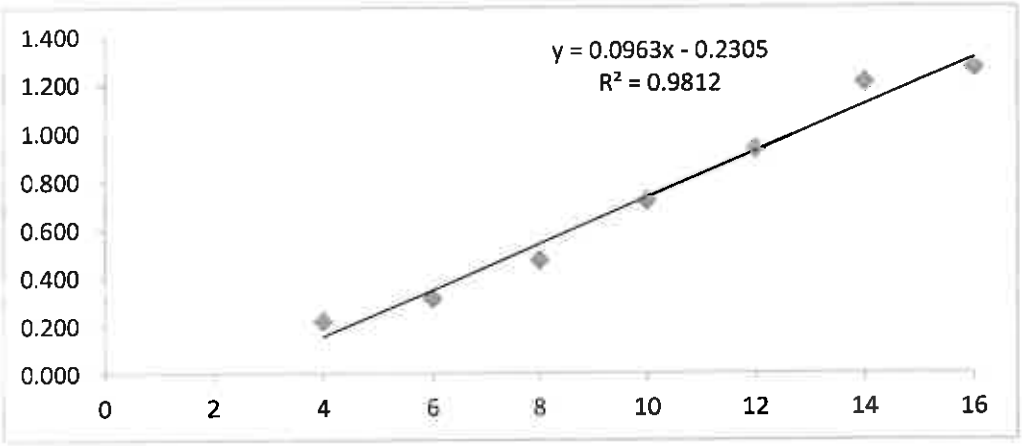


Tear	abs 1	abs 2	abs 3	average	% lacritin
P74 PRE	1.622	1.505	1.59	1.572	16.7
P74 1 DAY	1.764	1.721	1.798	1.761	19.0
P74 1 WEEK	1.751	1.7	1.712	1.721	18.5
P74 1 MO	1.658	1.727	1.671	1.685	18.0
P74 3 MO	1.741	1.783	1.737	1.754	18.9
P75 PRE	1.553	1.574	1.583	1.570	16.6
P751 DAY	1.755	1.809	1.756	1.773	19.1
P75 1 WEEK	1.834	1.879	1.825	1.846	20.0
P75 1 MO	1.74	1.838	1.889	1.822	19.7
P75 3 MO	1.805	1.879	1.978	1.887	20.5

ELISA - LASIK L01- L02 100 ng **5/23/13**

pLAC 3/20/13 = 244 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.237	1.283	1.278	1.266
14	1.186	1.233	1.203	1.207
12	0.905	0.932	0.957	0.931
10	0.703	0.725	0.727	0.718
8	0.459	0.479	0.478	0.472
6	0.292	0.366	0.284	0.314
4	0.217	0.219	0.216	0.217
2	0.18	0.178	0.173	0.177
0	0.136	0.133	0.12	0.130



Tear	abs 1	abs 2	abs 3	average	% lacritin
L01 PREOP	1.362	1.347	1.493	1.401	16.9
L01 1 DAY	1.35	1.307	1.279	1.312	16.0
L01 1 WK	1.729	1.736	1.735	1.733	20.4
L01 1 MO	1.406	1.352	1.486	1.415	17.1
L01 3 MO	1.479	1.453	1.458	1.463	17.6
L01 6 MO	1.157	1.196	1.181	1.178	14.6
L02 PREOP	1.243	1.25	1.239	1.244	15.3
L02 1 DAY	1.534	1.644	1.502	1.560	18.6
L02 1 MO	1.637	1.607	1.673	1.639	19.4
L02 3 MO	1.34	1.354	1.376	1.357	16.5
L02 6 MO	1.33	1.33	1.304	1.321	16.1

ELISA - LASIK L01-L02 100 ng **5/23/13**

pLAC 3/20/13 = 244 ug/mL

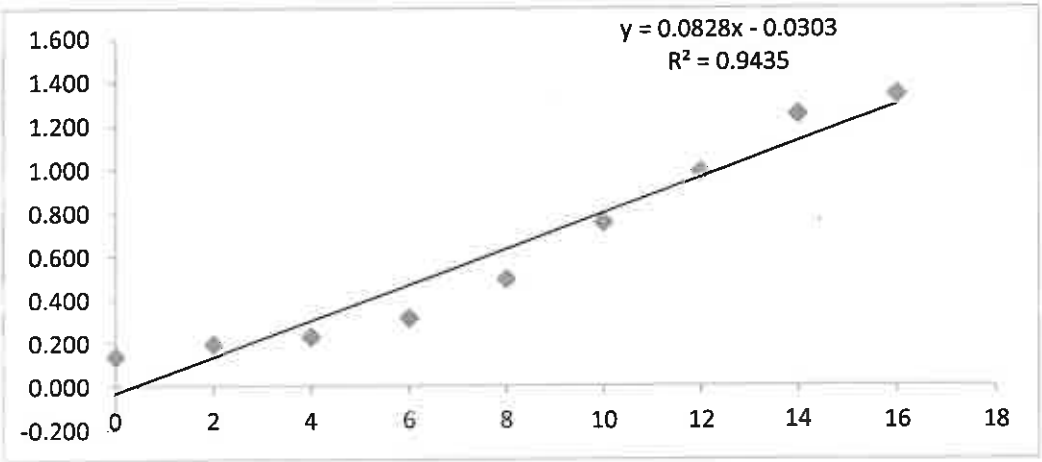
Blocking buffer = 1% BSA

Primary antibody = 1:400 Final Bleed 5529

Secondary antibody = 1:800 goat anti rabbit HRP

Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.339	1.309	1.366	1.338
14	1.249	1.234	1.26	1.248
12	0.979	0.976	1.007	0.987
10	0.749	0.755	0.754	0.753
8	0.491	0.49	0.496	0.492
6	0.301	0.289	0.349	0.313
4	0.223	0.226	0.23	0.226
2	0.208	0.184	0.186	0.193
0	0.136	0.142	0.132	0.137

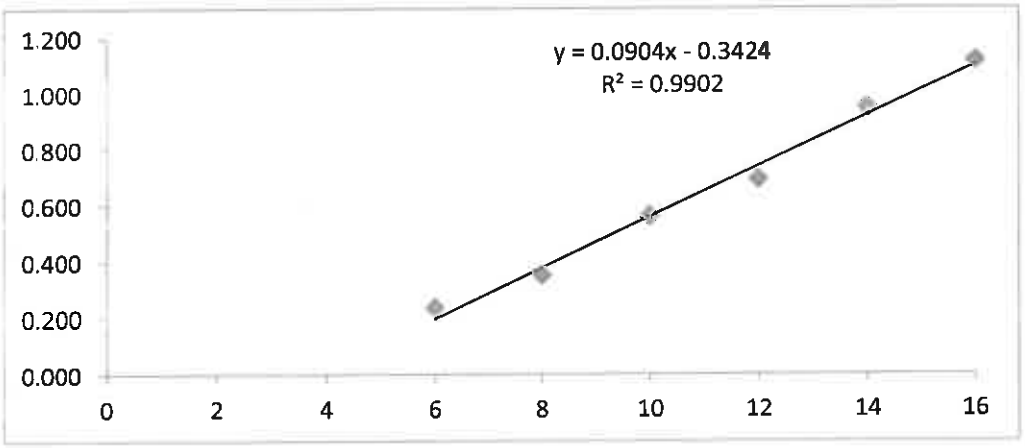


Tear	abs 1	abs 2	abs 3	average	% lacritin
L01 PREOP	1.385	1.377	1.347	1.370	16.9
L01 1 DAY	1.364	1.387	1.347	1.366	16.9
L01 1 WK	1.777	1.819	1.77	1.789	22.0
L01 1 MO	1.429	1.415	1.443	1.429	17.6
L01 3 MO	1.525	1.483	1.496	1.501	18.5
L01 6 MO	1.182	1.204	1.226	1.204	14.9
L02 PREOP	1.264	1.224	1.251	1.246	15.4
L02 1 DAY	1.546	1.603	1.57	1.573	19.4
L02 1 MO	1.689	1.686	1.693	1.689	20.8
L02 3 MO	1.446	1.391	1.367	1.401	17.3
L02 6 MO	1.375	1.308	1.396	1.360	16.8

ELISA - LASIK L03- L04 100 ng **5/22/13**

pLAC 3/20/13 = 248 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.081	1.119	1.153	1.118
14	0.964	0.926	0.961	0.950
12	0.678	0.693	0.704	0.692
10	0.583	0.512	0.589	0.561
8	0.353	0.349	0.356	0.353
6	0.246	0.231	0.234	0.237
4	0.216	0.222	0.209	0.216
2	0.178	0.17	0.174	0.174
0	0.167	0.161	0.152	

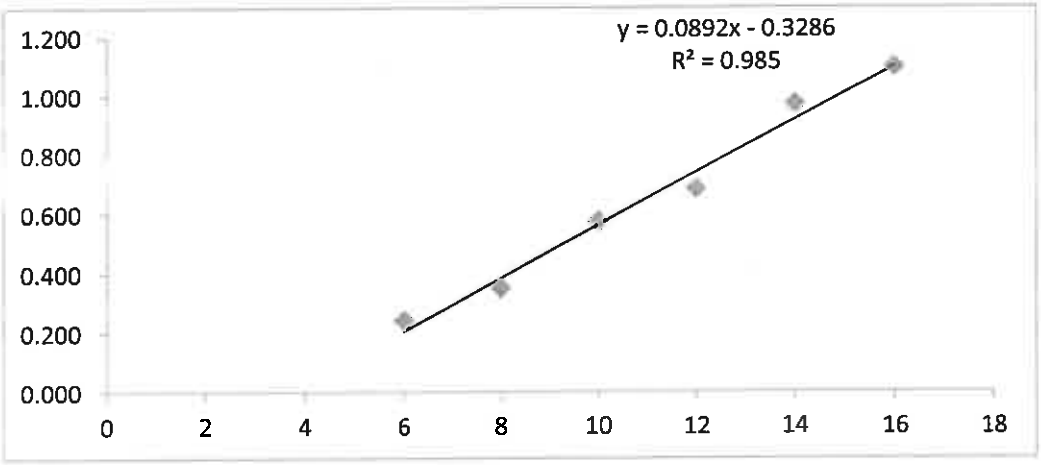


Tear	abs 1	abs 2	abs 3	average	% lacritin
L03 PREOP	1.185	1.067	1.071	1.108	16.0
L03 1 DAY	0.871	0.832	0.807	0.837	13.0
L03 1 MO	1.119	1.11	1.126	1.118	16.2
L03 3 MO	1.243	1.259	1.237	1.246	17.6
L03 6 MO	1.043	1.053	1.049	1.048	15.4
L04 PREOP	0.916	0.884	1.083	0.961	14.4
L04 1 DAY	0.676	0.663	0.681	0.673	11.2
L04 1 WEEK	0.627	0.646	0.662	0.645	10.9
L04 1 MO	0.463	0.472	0.457	0.464	8.9
L04 3 MO	0.59	0.579	0.58	0.583	10.2
L04 6 MO	0.729	0.586	0.693	0.669	11.2

ELISA - LASIK L03-L04 100 ng **5/22/13**

pLAC 3/20/13 = 248 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.076	1.126	1.09	1.097
14	0.975	0.975	0.963	0.971
12	0.678	0.692	0.681	0.684
10	0.563	0.573	0.584	0.573
8	0.351	0.35	0.352	0.351
6	0.247	0.238	0.241	0.242
4	0.223	0.22	0.236	0.226
2	0.179	0.172	0.184	0.178
0	0.16	0.163	0.16	

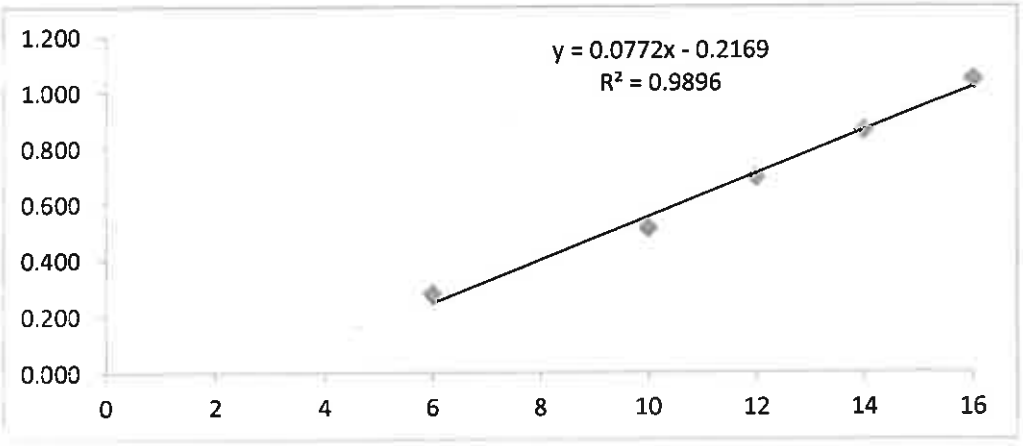


Tear	abs 1	abs 2	abs 3	average	% lacritin
L03 PREOP	1.025	1.014	1.042	1.027	15.2
L03 1 DAY	0.87	0.807	0.916	0.864	13.4
L03 1 MO	1.12	1.098	1.119	1.112	16.2
L03 3 MO	1.217	1.191	1.174	1.194	17.1
L03 6 MO	0.984	1.023	1.012	1.006	15.0
L04 PREOP	0.857	0.845	0.838	0.847	13.2
L04 1 DAY	0.675	0.694	0.667	0.679	11.3
L04 1 WEEK	0.606	0.628	0.624	0.619	10.6
L04 1 MO	0.489	0.485	0.477	0.484	9.1
L04 3 MO	0.569	0.613	0.552	0.578	10.2
L04 6 MO	0.689	0.724	0.705	0.706	11.6

ELISA - LASIK L05 - L06 100 ng **5/24/13**

pLAC 3/20/13 = 250 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.011	1.063	1.062	1.045
14	0.857	0.852	0.875	0.861
12	0.695	0.692	0.696	0.694
10	0.501	0.52	0.518	0.513
6	0.281	0.273	0.279	0.278
4	0.241	0.249	0.244	0.245
2	0.185	0.17	0.175	0.177
0	0.179	0.144	0.152	0.158

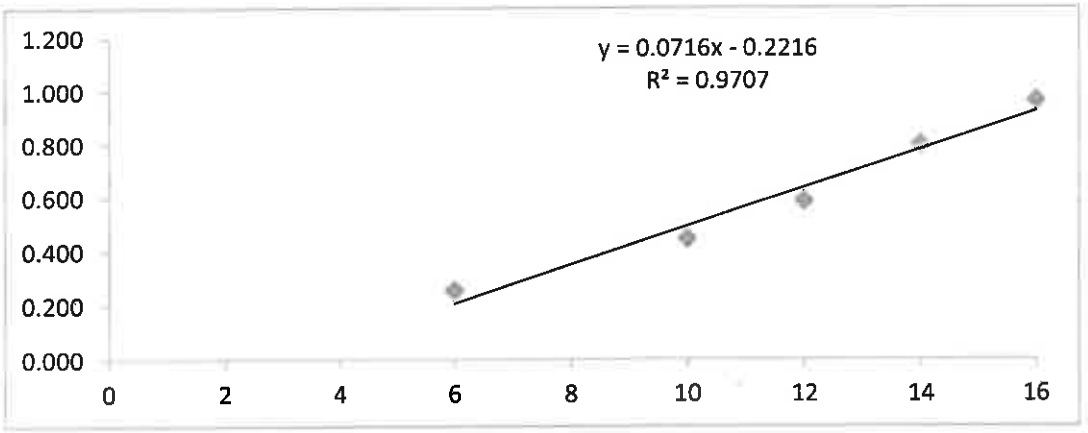


Tear	abs 1	abs 2	abs 3	average	% lacritin
L05 PRE	0.576	0.58	0.629	0.595	10.5
L05 1 DAY	0.962	0.991	0.974	0.976	15.4
L05 1 WEEK	0.794	0.799	0.805	0.799	13.2
L05 1 MO	1.095	1.132	1.146	1.124	17.4
L05 3 MO	0.785	0.794	0.798	0.792	13.1
L05 6 MO	1.263	1.273	1.268	1.268	19.2
L06 PRE	0.838	0.826	0.85	0.838	13.7
L06 1 DAY	0.532	0.528	0.535	0.532	9.7
L06 1 WEEK	0.917	0.893	0.912	0.907	14.6
L06 1 MO	0.547	0.54	0.535	0.541	9.8
L06 3 MO	0.772	0.813	0.855	0.813	13.3
L06 6 MO	0.547	0.588	0.553	0.563	10.1

ELISA - LASIK L05 - L06 100 ng **5/24/13**

pLAC 3/20/13 = 250 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	0.956	0.956	0.977	0.963
14	0.806	0.797	0.789	0.797
12	0.578	0.59	0.589	0.586
10	0.443	0.438	0.455	0.445
6	0.264	0.25	0.253	0.256
4	0.207	0.199	0.2	0.202
2	0.167	0.164	0.157	0.163
0	0.142	0.134	0.138	0.138

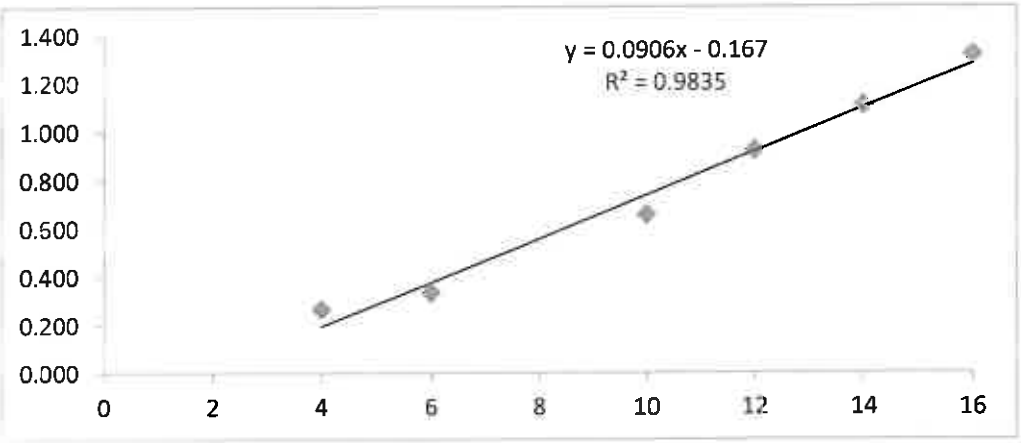


Tear	abs 1	abs 2	abs 3	average	% lacritin
L05 PRE	0.577	0.58	0.585	0.581	11.2
L05 1 DAY	0.956	0.962	0.923	0.947	16.3
L05 1 WEEK	0.784	0.801	0.789	0.791	14.1
L05 1 MO	1.09	1.125	1.067	1.094	18.4
L05 3 MO	0.809	0.804	0.795	0.803	14.3
L05 6 MO	1.246	1.23	1.24	1.239	20.4
L06 PRE	0.805	0.864	0.809	0.826	14.6
L06 1 DAY	0.535		0.518	0.527	10.4
L06 1 WEEK	0.687	0.902	0.893	0.827	14.6
L06 1 MO	0.468	0.521	0.486	0.492	10.0
L06 3 MO	0.741	0.756	0.783	0.760	13.7
L06 6 MO	0.505	0.534	0.499	0.513	10.3

ELISA - LASIK L07 - L08 100 ng **5/28/13**

pLAC 3/20/13 = 250 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.399	1.289	1.278	1.322
14	1.144	1.092	1.09	1.109
12	0.93	0.926	0.913	0.923
10	0.659	0.669	0.648	0.659
6	0.352	0.33	0.328	0.337
4	0.276	0.268	0.257	0.267
2	0.253	0.198	0.194	0.215
0	0.165	0.158	0.157	0.160

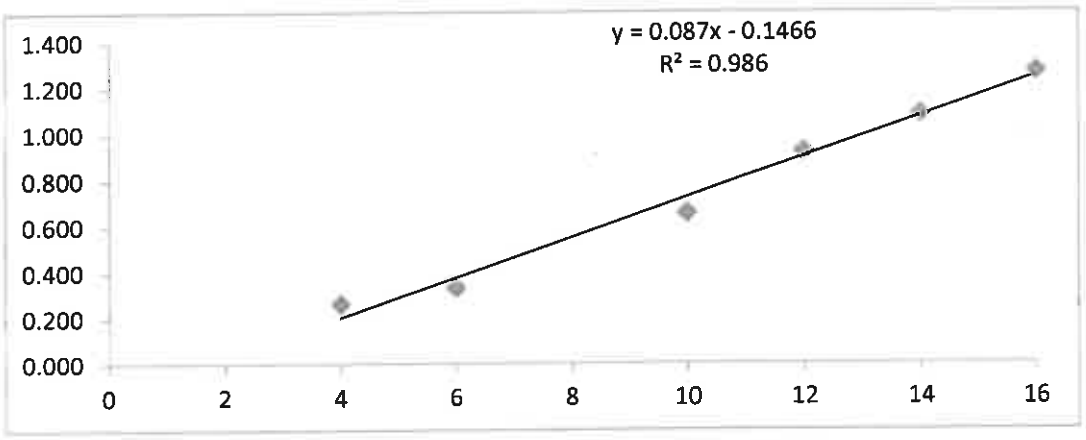


Tear	abs 1	abs 2	abs 3	average	% lacritin
L07 PRE	0.408	0.417	0.414	0.413	6.4
L07 1 DAY	1.162	1.183	1.152	1.166	14.7
L07 1 WEEK	1.091	1.153	1.159	1.134	14.4
L07 1 MO	0.863	0.867	0.954	0.895	11.7
L07 3 MO	1.291	1.253	1.301	1.282	16.0
L07 6 MO	1.006	1.033	0.982	1.007	13.0
L08 PRE	0.604	0.584	0.592	0.593	8.4
L08 1 DAY	0.626	0.648	0.639	0.638	8.9
L08 1 WEEK	0.549	0.569	0.555	0.558	8.0
L08 1 MO	0.443	0.442	0.456	0.447	6.8
L08 3 MO	0.642	0.663	0.67	0.658	9.1
L08 6 MO		1.013	0.931	0.972	12.6

ELISA - LASIK L07 - L08 100 ng **5/28/13**

pLAC 3/20/13 = 250 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.266	1.258	1.268	1.264
14	1.095	1.074	1.075	1.081
12	0.939	0.88	0.939	0.919
10	0.653	0.67	0.641	0.655
6	0.326	0.336	0.331	0.331
4	0.266	0.267	0.258	0.264
2	0.195	0.202	0.2	0.199
0	0.241	0.159	0.159	0.186

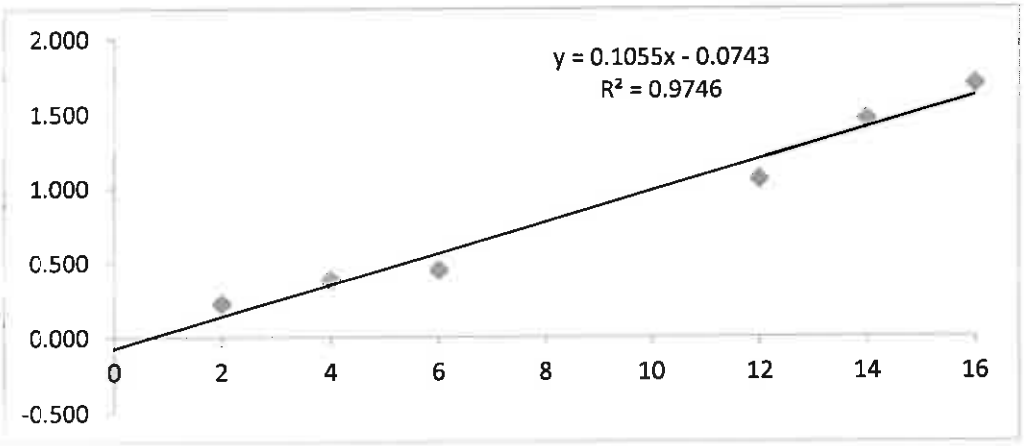


Tear	abs 1	abs 2	abs 3	average	% lacritin
L07 PRE	0.425	0.429	0.414	0.423	6.5
L07 1 DAY	1.132	1.151	1.149	1.144	14.8
L07 1 WEEK	1.068	1.112	1.133	1.104	14.4
L07 1 MO	0.899	0.882	0.924	0.902	12.0
L07 3 MO	1.273	1.29	1.277	1.280	16.4
L07 6 MO	1.008	1.006	1.061	1.025	13.5
L08 PRE	0.611	0.575	0.591	0.592	8.5
L08 1 DAY	0.629	0.648	0.633	0.637	9.0
L08 1 WEEK	0.581	0.589	0.579	0.583	8.4
L08 1 MO	0.461	0.462	0.478	0.467	7.1
L08 3 MO	0.61	0.627	0.65	0.629	8.9
L08 6 MO	0.951		1.027	0.989	13.1

ELISA - LASIK L10 - L11 100 ng **5/31/13**

pLAC 3/20/13 = 250 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.717	1.688	1.672	1.692
14	1.462	1.487	1.42	1.456
12	1.028	1.079	1.062	1.056
10	0.758	0.768	0.767	
6	0.452	0.444	0.443	0.446
4	0.394	0.377	0.366	0.379
2	0.229	0.216	0.219	0.221
0	0.2	0.188	0.191	

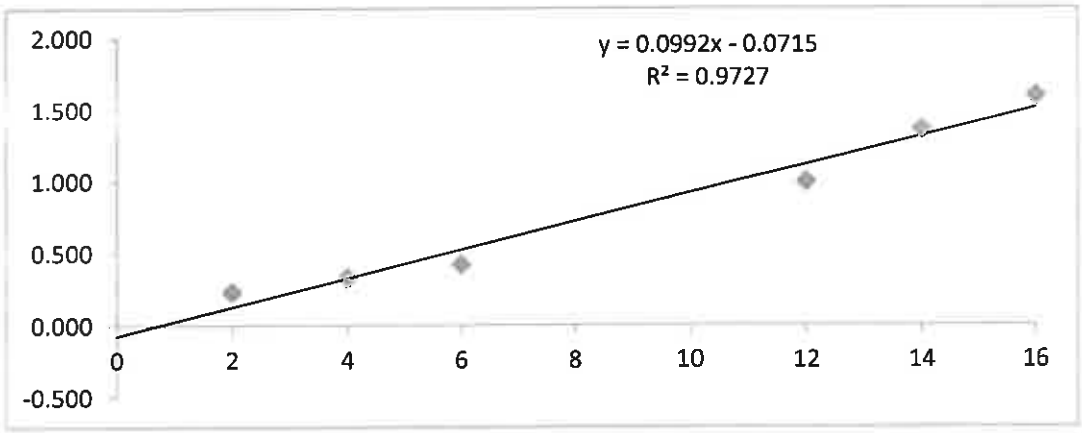


Tear	abs 1	abs 2	abs 3	average	% lacritin
L10 PRE	1.364	1.419	1.41	1.398	14.0
L10 1 DAY	1.643	1.648	1.66	1.650	16.3
L10 1 WEEK	1.568	1.605	1.583	1.585	15.7
L10 1 MO	1.76	1.7	1.819	1.760	17.4
L10 3 MO	0.476	0.487	0.484	0.482	5.3
L10 6 MO	1.66	1.63	1.676	1.655	16.4
L11 PRE	1.338	1.319	1.311	1.323	13.2
L11 1 DAY	1.097	1.128	1.145	1.123	11.4
L11 1 WEEK	1.688	1.72	1.729	1.712	16.9
L11 1 MO	1.212	1.185	1.204	1.200	12.1
L11 3 MO	1.506	1.545	1.521	1.524	15.1
L11 6 MO	1.415	1.343	1.355	1.371	13.7

ELISA - LASIK L10 - L11 100 ng **5/31/13**

pLAC 3/20/13 = 250 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.575	1.595	1.621	1.597
14	1.340	1.401	1.344	1.362
12	0.93	1.028	1.023	0.994
10	0.708	0.727	0.72	
6	0.44	0.409	0.409	0.419
4	0.335	0.324	0.32	0.326
2	0.232	0.236	0.222	0.230
0	0.196	0.182	0.192	

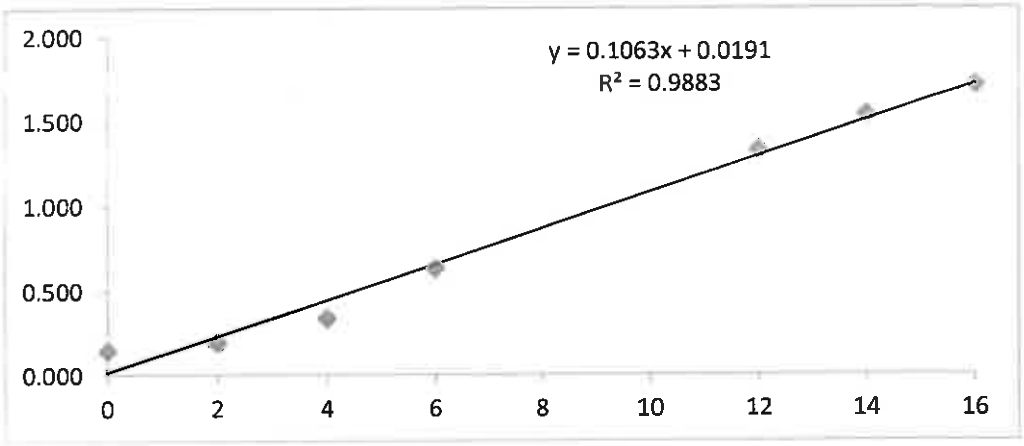


Tear	abs 1	abs 2	abs 3	average	% lacritin
L10 PRE	1.306	1.37	1.407	1.361	14.4
L10 1 DAY	1.529	1.597	1.536	1.554	16.4
L10 1 WEEK	1.417	1.48	1.478	1.458	15.4
L10 1 MO	1.59	1.638	1.562	1.597	16.8
L10 3 MO	0.478	0.444	0.433	0.452	5.3
L10 6 MO	1.554	1.553	1.586	1.564	16.5
L11 PRE	1.229	1.227	1.244	1.233	13.2
L11 1 DAY	1.059	1.063	1.054	1.059	11.4
L11 1 WEEK	1.599	1.66	1.68	1.646	17.3
L11 1 MO	1.106	1.155	1.158	1.140	12.2
L11 3 MO	1.399	1.457	1.401	1.419	15.0
L11 6 MO	1.284	1.328	1.296	1.303	13.9

ELISA - LASIK L12, L50 100 ng **6/4/13**

pLAC 4/26/13 = 250 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.754	1.737	1.652	1.714
14	1.543	1.506	1.557	1.535
12	1.337	1.316	1.322	1.325
10	0.9	0.886	0.917	
6	0.541	0.56	0.789	0.630
4	0.353	0.321	0.332	0.335
2	0.202	0.183	0.184	0.190
0	0.145	0.137	0.147	0.143

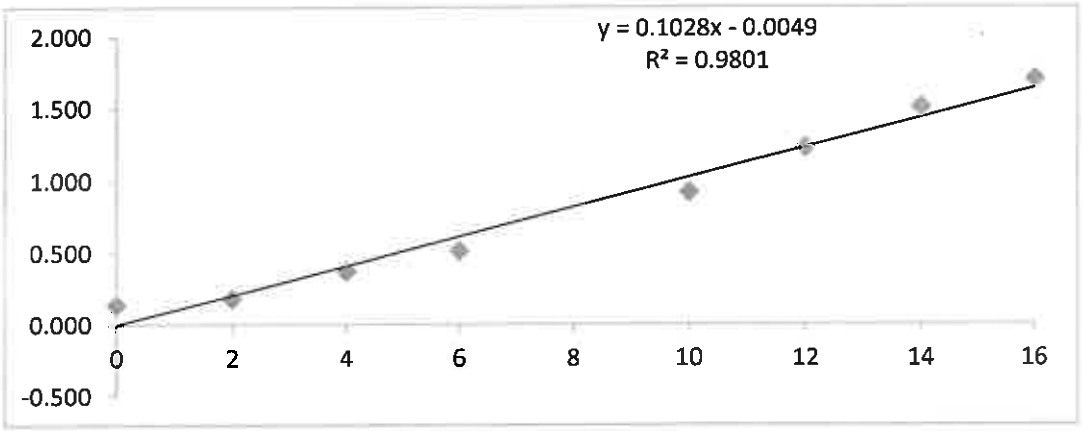


Tear	abs 1	abs 2	abs 3	average	% lacritin
L12 PREOP	0.933	1.047	0.866	0.949	8.7
L12 1 DAY	2.215	1.915	1.502	1.877	17.5
L12 1 WK	1.271	1.595	1.236	1.367	12.7
L12 1 MO	1.15	1.168	1.157	1.158	10.7
L12 3 MO	1.187	1.217	1.216	1.207	11.2
L12 6 MO	1.031	1.014	1.021	1.022	9.4
L50 PREOP	0.956	1.248	1.328	1.177	10.9
L50 1 DAY	0.835	0.737	0.859	0.810	7.4
L50 1 WK	1.804	1.238	1.338	1.460	13.6
L50 1 MO	1.456	1.497	1.534	1.496	13.9
L50 3 MO	1.134	1.232	1.223	1.196	11.1
L50 6 MO	0.765	0.735	0.816	0.772	7.1

ELISA - LASIK L12, L50 100 ng **6/4/13**

pLAC 4/26/13 = 250 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

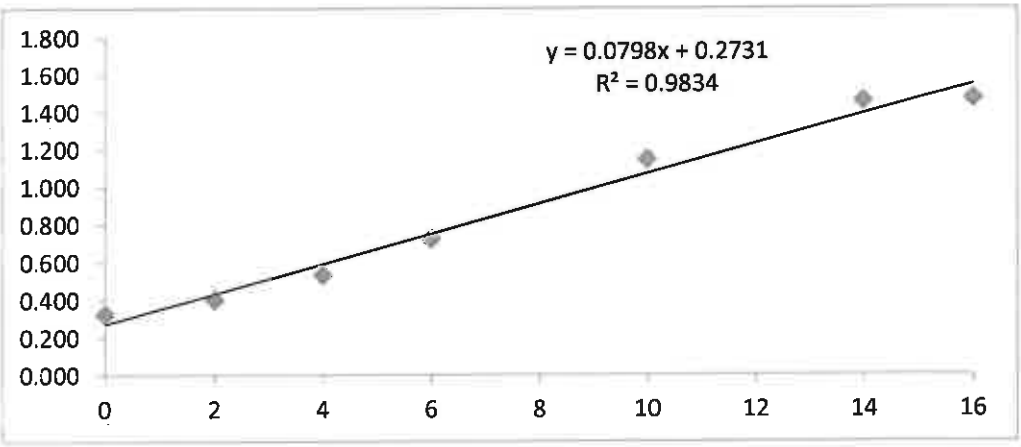
2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.711	1.698	1.69	1.700
14	1.516	1.48	1.511	1.502
12	1.196	1.293	1.189	1.226
10	0.904	0.944	0.906	0.918
6	0.527	0.503	0.507	0.512
4	0.338	0.371	0.398	0.369
2	0.178	0.17	0.184	0.177
0	0.131	0.136	0.147	0.138



Tear	abs 1	abs 2	abs 3	average	% lacritin
L12 PREOP	0.911	0.844	0.866	0.874	8.5
L12 1 DAY	1.461	1.446	1.502	1.470	14.3
L12 1 WK	1.155	1.228	1.236	1.206	11.8
L12 1 MO	1.146	1.161	1.157	1.155	11.3
L12 3 MO	1.235	1.167	1.24	1.214	11.9
L12 6 MO	1.011	0.997	1.006	1.005	9.8
L50 PREOP	1.005	0.816	1.005	0.942	9.2
L50 1 DAY	0.813	0.725	0.738	0.759	7.4
L50 1 WK	1.388	1.367	1.346	1.367	13.3
L50 1 MO	1.45	1.448	1.482	1.460	14.3
L50 3 MO	1.121	1.218	1.223	1.187	11.6
L50 6 MO	0.841	0.759	0.761	0.787	7.7

ELISA - LASIK L14 - L15 100 ng 8/23/13
pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.479	1.475	1.462	1.472
14	1.391	1.467	1.515	1.458
12	1.305	1.43	1.461	
10	1.126	1.152	1.157	1.145
6	0.707	0.707	0.758	0.724
4	0.546	0.515	0.541	0.534
2	0.367	0.388	0.452	0.402
0	0.325	0.33	0.317	0.324



Tear	abs 1	abs 2	abs 3	average	% lacritin
L14 PRE	1.495	1.389	1.464	1.449	14.3
L14 1 DAY	1.456	1.555	1.426	1.479	14.6
L14 1 WEEK	1.576	1.535	1.47	1.527	15.2
L14 1 MO	1.614	1.481	1.463	1.519	15.1
L14 3 MO	1.67	1.558	1.548	1.592	16.0
L14 6 MO	1.75	1.731	1.796	1.759	18.0
L15 PRE	1.74	1.727	1.61	1.692	17.2
L15 1 DAY	1.837	1.832	1.711	1.793	18.4
L15 1 WEEK	1.806	1.723	1.913	1.814	18.7
L15 1 MO	1.634	1.646	1.775	1.685	17.1
L15 3 MO	1.524	1.564	1.576	1.555	15.5
L15 6 MO	1.374	1.421	1.637	1.477	14.6

ELISA - LASIK L14 - L15 100 ng 8/23/13

pLAC 5/29/13 = 321 ug/mL

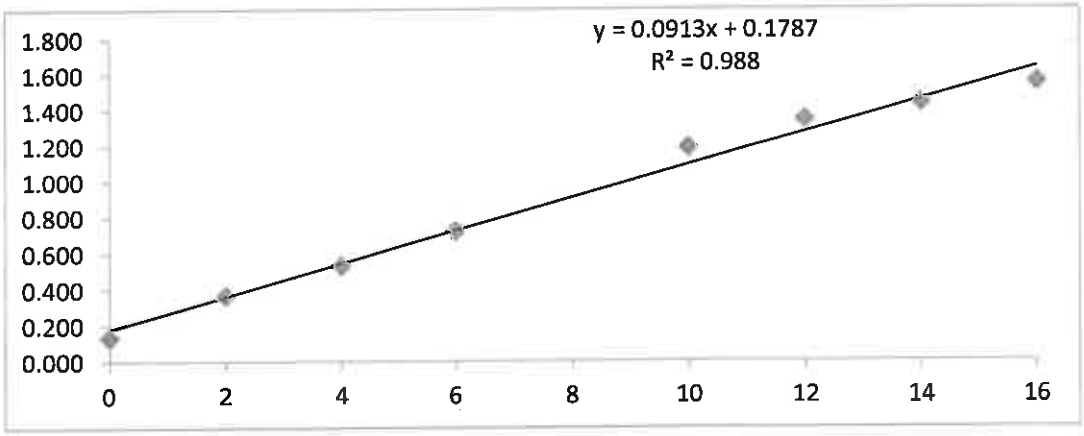
Blocking buffer = 1% BSA

Primary antibody = 1:400 Final Bleed 5529

Secondary antibody = 1:800 goat anti rabbit HRP

Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.574	1.551	1.526	1.550
14	1.472	1.456	1.38	1.436
12	1.333	1.378	1.316	1.342
10	1.184	1.222	1.155	1.187
6	0.72	0.699	0.749	0.723
4	0.547	0.519	0.528	0.531
2	0.365	0.38	0.358	0.368
0	0.137	0.134	0.133	0.135

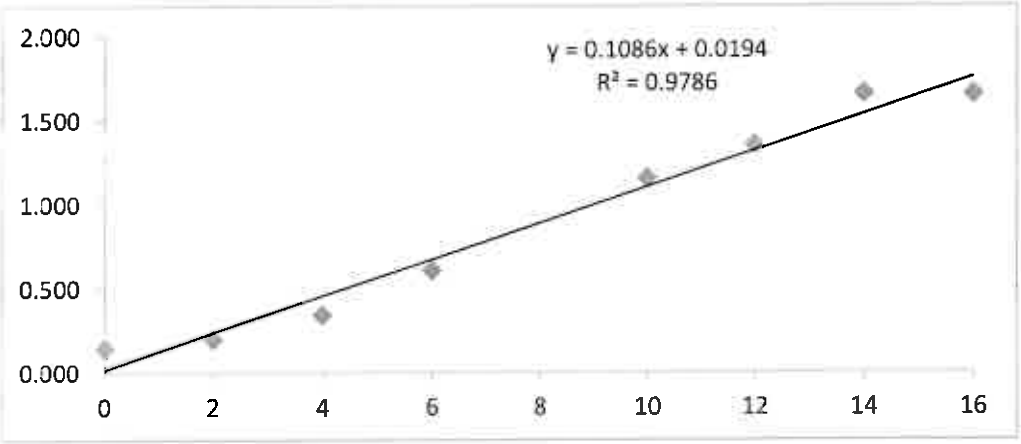


Tear	abs 1	abs 2	abs 3	average	% lacritin
L14 PRE	1.416	1.397	1.384	1.399	13.4
L14 1 DAY	1.404	1.343	1.439	1.395	13.3
L14 1 WEEK	1.52	1.48	1.541	1.514	14.6
L14 1 MO	1.662	1.554	1.666	1.627	15.9
L14 3 MO	1.648	1.653	1.667	1.656	16.2
L14 6 MO	1.67	1.68	1.747	1.699	16.7
L15 PRE	1.648	1.66	1.712	1.673	16.4
L15 1 DAY	1.735	1.736	1.741	1.737	17.1
L15 1 WEEK	1.828	1.852	1.955	1.878	18.6
L15 1 MO	1.727	1.77	1.858	1.785	17.6
L15 3 MO	1.631	1.677	1.657	1.655	16.2
L15 6 MO	1.446	1.478	1.574	1.499	14.5

ELISA - LASIK P18-P19 100 ng **7/2/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.63	1.681	1.658	1.656
14	1.642	1.632	1.703	1.659
12	1.33	1.377	1.361	1.356
10	1.127	1.169	1.17	1.155
6	0.611	0.585	0.62	0.605
4	0.337	0.316	0.367	0.340
2	0.206	0.184	0.193	0.194
0	0.145	0.135	0.144	0.141

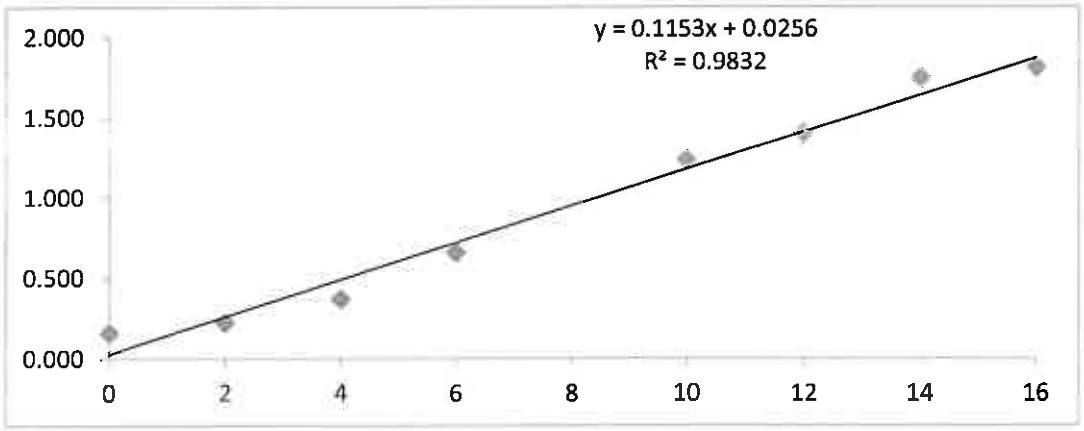


Tear	abs 1	abs 2	abs 3	average	% lacritin
P18 PRE	1.214	1.175	1.189	1.193	10.8
P18 1 DAY	1.054	1.095	1.071	1.073	9.7
P18 1 WEEK	1.171	1.177	1.146	1.165	10.5
P18 1 MO	0.827	0.813	0.826	0.822	7.4
P18 3 MO	1.148	1.194	1.148	1.163	10.5
P18 6 MO	1.266	1.269	1.285	1.273	11.5
P19 PRE	1.366	1.405	1.354	1.375	12.5
P19 1 DAY	0.969	0.99	0.988	0.982	8.9
P19 1 WEEK	1.53	1.554	1.599	1.561	14.2
P19 1 MO	1.495	1.553	1.544	1.531	13.9
P19 3 MO	1.968	1.957	1.977	1.967	17.9
P19 6 MO	1.408	1.574	1.481	1.488	13.5

ELISA - LASIK P18 - P19 100 ng **7/2/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.783	1.804	1.837	1.808
14	1.738	1.739	1.758	1.745
12	1.378	1.4	1.426	1.401
10	1.175	1.272	1.257	1.235
6	0.634	0.664	0.674	0.657
4	0.359	0.364	0.372	0.365
2	0.217	0.221	0.222	0.220
0	0.16	0.152	0.151	0.154

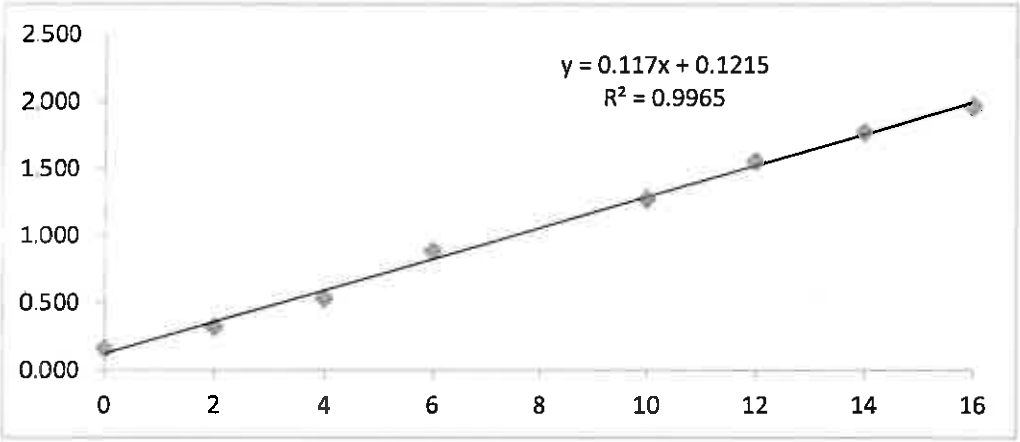


Tear	abs 1	abs 2	abs 3	average	% lacritin
P18 PRE	1.307	1.334	1.315	1.319	11.2
P18 1 DAY	1.358	1.352	1.377	1.362	11.6
P18 1 WEEK	0.985	0.954	0.916	0.952	8.0
P18 1 MO	1.352	1.325	1.309	1.329	11.3
P18 3 MO	1.43	1.418	1.442	1.430	12.2
P18 6 MO	1.3	1.324	1.313	1.312	11.2
P19 PRE	1.463	1.454	1.433	1.450	12.4
P19 1 DAY	0.975	1.018	0.929	0.974	8.2
P19 1 WEEK	1.593	1.661	1.652	1.635	14.0
P19 1 MO	1.508	1.542	1.548	1.533	13.1
P19 3 MO	1.978	2.024	2.03	2.011	17.2
P19 6 MO	1.532	1.551	1.459	1.514	12.9

ELISA - LASIK L51, L52 100 ng **6/20/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.959	1.955	1.992	1.969
14	1.780	1.767	1.771	1.773
12	1.523	1.558	1.585	1.555
10	1.243	1.255	1.329	1.276
6	0.861	0.883	0.904	0.883
4	0.529	0.539	0.526	0.531
2	0.328	0.317	0.307	0.317
0	0.165	0.154	0.158	0.159

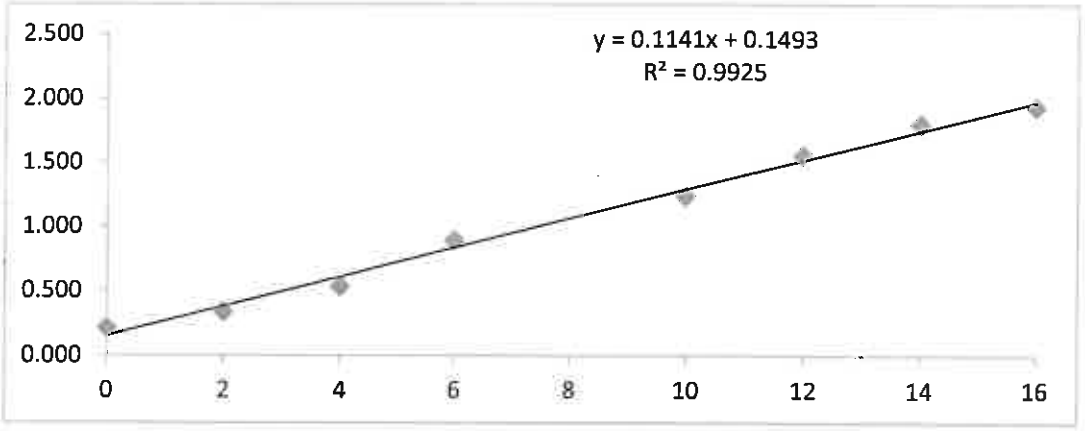


Tear	abs 1	abs 2	abs 3	average	% lacritin
L51 PREOP	1.389	1.387	1.345	1.374	10.7
51 1 DAY	1.415	1.428	1.497	1.447	11.3
L51 1 WK	1.463	1.496	1.496	1.485	11.7
L51 1 MO	1.583	1.585	1.587	1.585	12.5
L51 3 MO	1.612	1.561	1.612	1.595	12.6
L51 6 MO	1.054	1.095	1.048	1.066	8.1
L52 PREOP	1.579	1.637	1.636	1.617	12.8
L52 1 DAY	1.641	1.568	1.567	1.592	12.6
L52 1 WK	1.728	1.848	1.68	1.752	13.9
L52 1 MO	1.257	1.553	1.549	1.453	11.4
L52 3 MO	0.141	0.128	0.133	0.134	0.1
L52 6 MO	2.131	2.177	2.121	2.143	17.3

ELISA - LASIK L51, L52 100 ng **6/20/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.906	2.03	1.873	1.936
14	1.722	1.831	1.851	1.801
12	1.527	1.609	1.53	1.555
10	1.214	1.245	1.238	1.232
6	0.878	0.902	0.892	0.891
4	0.528	0.553	0.513	0.531
2	0.344	0.34	0.32	0.335
0	0.177	0.248	0.211	0.212

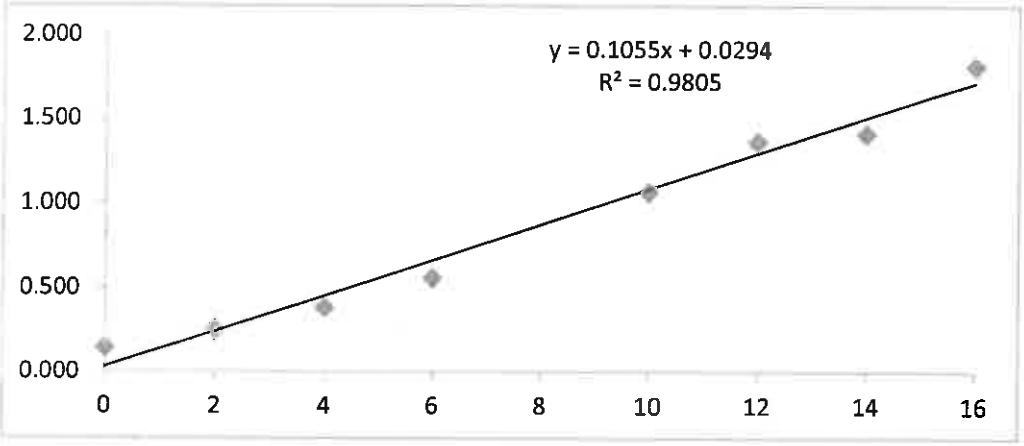


Tear	abs 1	abs 2	abs 3	average	% lacritin
L51 PREOP	1.398	1.426	1.439	1.421	11.1
51 1 DAY	1.448	1.535	1.538	1.507	11.9
L51 1 WK	1.468	1.58	1.567	1.538	12.2
L51 1 MO	1.678	1.667	1.553	1.633	13.0
L51 3 MO	1.63	1.62	1.677	1.642	13.1
L51 6 MO	1.13	1.127	1.115	1.124	8.5
L52 PREOP	1.639	1.762	1.813	1.738	13.9
L52 1 DAY	1.656	1.571	1.672	1.633	13.0
L52 1 WK	1.696	1.796	1.817	1.770	14.2
L52 1 MO	1.6	1.593	1.519	1.571	12.5
L52 3 MO	0.176	0.171	0.177	0.175	0.2
L52 6 MO	2.156	2.093	2.147	2.132	17.4

ELISA - LASIK L53, L54 100 ng **6/26/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.799	1.817	1.836	1.817
14	1.408	1.406	1.419	1.411
12	1.38	1.361	1.353	1.365
10	1.064	1.058	1.074	1.065
6	0.556	0.536	0.582	0.558
4	0.388	0.374	0.384	0.382
2	0.257	0.238	0.242	0.246
0	0.146	0.144	0.142	0.144

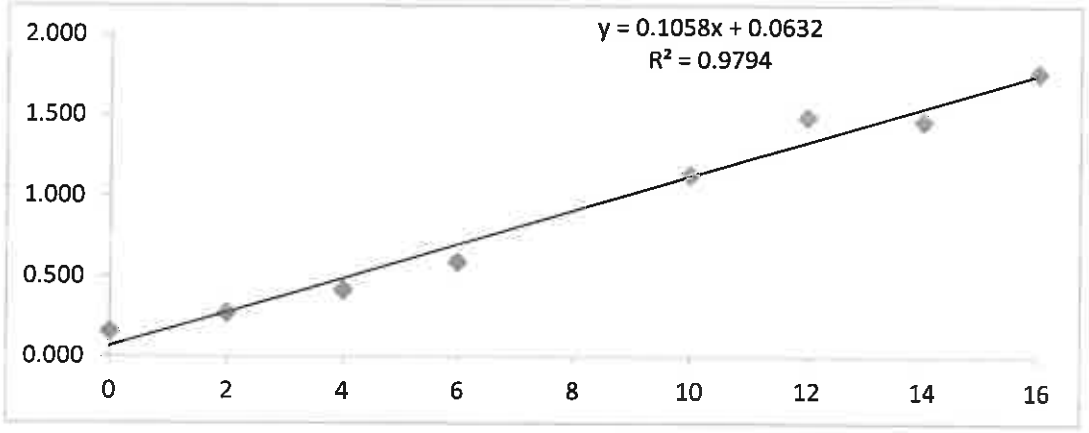


Tear	abs 1	abs 2	abs 3	average	% lacritin
L53 PREOP	1.232	1.235	1.212	1.226	11.3
53 1 DAY	1.425	1.446	1.401	1.424	13.2
L53 1 WK	1.433	1.372	1.329	1.378	12.8
L53 1 MO	1.218	1.146	1.105	1.156	10.7
L53 3 MO	1.256	1.206	1.197	1.220	11.3
L53 6 MO	1.364	1.341	1.363	1.356	12.6
L54 PREOP	1.597	1.553	1.547	1.566	14.6
L54 1 DAY	1.518	1.563	1.527	1.536	14.3
L54 1 WK	1.683	1.759	1.762	1.735	16.2
L54 1 MO	0.829	0.836	0.896	0.854	7.8
L54 3 MO	1.185	1.132	1.132	1.150	10.6
L54 6 MO	1.195	1.174	1.177	1.182	10.9

ELISA - LASIK L53, L54 100 ng **6/26/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.704	1.769	1.822	1.765
14	1.355	1.531	1.499	1.462
12	1.36	1.52	1.584	1.488
10	1.09	1.222	1.081	1.131
6	0.588	0.549	0.63	0.589
4	0.414	0.407	0.425	0.415
2	0.291	0.267	0.258	0.272
0	0.155	0.155	0.323	0.155

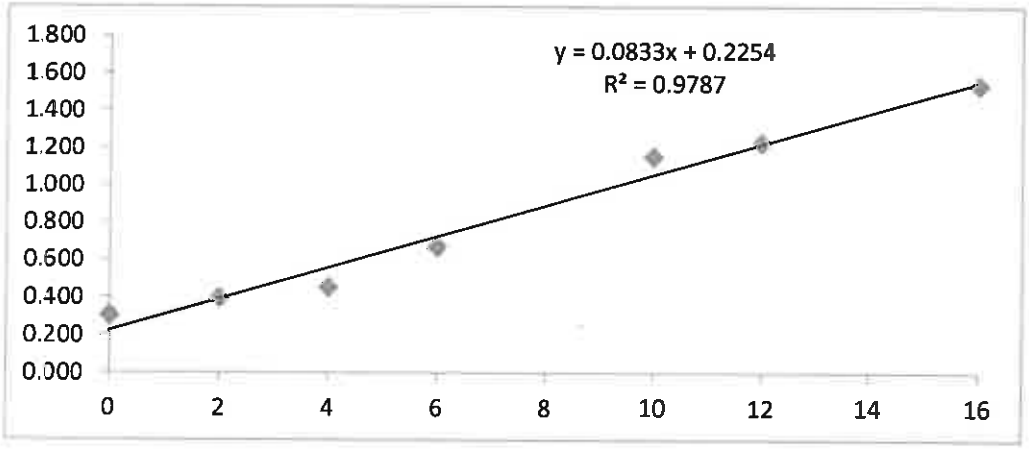


Tear	abs 1	abs 2	abs 3	average	% lacritin
L53 PREOP	1.22	1.36	1.359	1.313	11.8
53 1 DAY	1.425	1.617	1.493	1.512	13.7
L53 1 WK	1.433	1.435	1.403	1.424	12.9
L53 1 MO	1.107	1.19	1.147	1.148	10.3
L53 3 MO	1.182	1.247	1.297	1.242	11.1
L53 6 MO	1.488	1.466	1.396	1.450	13.1
L54 PREOP	1.721	1.549	1.545	1.605	14.6
L54 1 DAY	1.619	1.511	1.515	1.548	14.0
L54 1 WK	1.772	1.738	1.724	1.745	15.9
L54 1 MO	0.846	0.901	0.921	0.889	7.8
L54 3 MO	1.182	1.154	1.132	1.156	10.3
L54 6 MO	1.12	1.172	1.181	1.158	10.3

ELISA - LASIK L55, L13 100 ng **8/30/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

1 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.557	1.552	1.516	1.542
14	1.580	1.536	1.539	
12	1.293	1.273	1.126	1.231
10	1.22	1.112	1.126	1.153
6	0.67	0.655	0.677	0.667
4	0.47	0.513	0.37	0.451
2	0.371	0.376	0.437	0.395
0	0.321	0.283	0.303	0.302

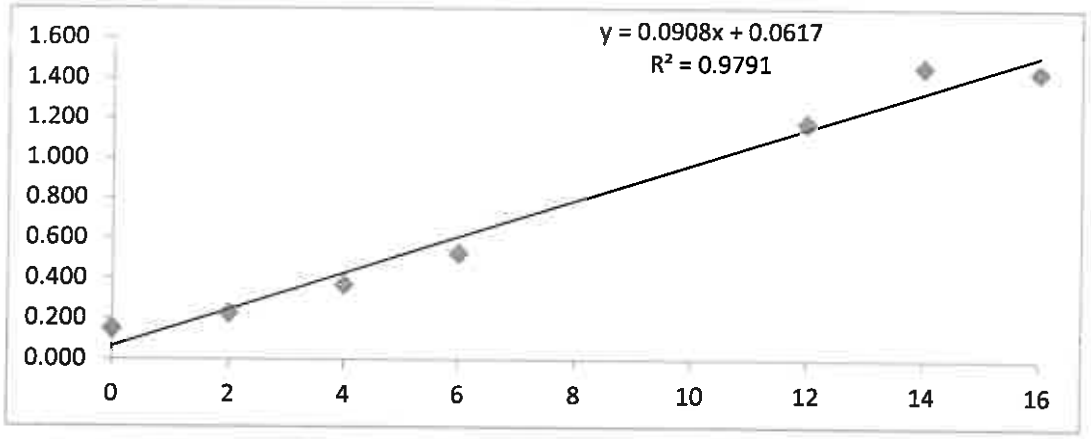


Tear	abs 1	abs 2	abs 3	average	% lacritin
L55 PREOP	1.458	1.434	1.301	1.398	13.6
L55 1 DAY	1.624	1.59	1.778	1.664	16.6
L55 1 WK	1.852	1.807	1.653	1.771	17.8
L55 1 MO	1.924	1.879	2.001	1.935	19.7
L55 3 MO	1.726	1.767	1.795	1.763	17.7
L55 6 MO	1.805	1.802	1.798	1.802	18.2
L13 PREOP	1.63	1.632	1.628	1.630	16.2
L13 1 DAY	1.595	1.677	1.702	1.658	16.5
L13 1 WK	1.622	1.757	1.797	1.725	17.3
L13 1 MO	1.82	1.803	1.883	1.835	18.6
L13 3 MO	1.626	1.713	1.683	1.674	16.7
L13 6 MO	1.213	1.36	1.292	1.288	12.3

ELISA - LASIK L55, L13 100 ng **8/30/13**

pLAC 5/29/13 = 321 ug/mL
Blocking buffer = 1% BSA
Primary antibody = 1:400 Final Bleed 5529
Secondary antibody = 1:800 goat anti rabbit HRP
Substrate incubation = 10 minutes

2 pLAC (ng)	abs 1	abs 2	abs 3	average
16	1.429	1.436	1.434	1.433
14	1.469	1.443	1.465	1.459
12	1.146	1.163	1.223	1.177
10	1.145	1.149	1.164	
6	0.506	0.542	0.524	0.524
4	0.344	0.377	0.382	0.368
2	0.222	0.203	0.251	0.225
0	0.162	0.138	0.216	0.150



Tear	abs 1	abs 2	abs 3	average	% lacritin
L55 PREOP	1.625	1.646	1.523	1.598	16.9
L55 1 DAY	1.725	1.667	1.655	1.682	17.8
L55 1 WK	1.783	1.715	1.791	1.763	18.7
L55 1 MO	1.845	1.751	1.455	1.684	17.9
L55 3 MO	1.759	1.865	1.793	1.806	19.2
L55 6 MO	1.759	1.84	1.821	1.807	19.2
L13 PREOP	1.644	1.696	1.566	1.635	17.3
L13 1 DAY	1.54	1.548	1.581	1.556	16.5
L13 1 WK	1.603	1.74	1.718	1.687	17.9
L13 1 MO	1.709	1.798	1.854	1.787	19.0
L13 3 MO	1.801	1.759	1.703	1.754	18.6
L13 6 MO	1.312	1.246	1.292	1.283	13.5